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Nutrition as a Factor against Addiction*

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HUNGER, which is the central nervous sensation resulting from starvation, has deep psychological consequences, not only in its acute but also in its chronic form.

The characteristic signs of deterioration of the personality in semistarvation have been described by Keys and Brožek as a lack of spontaneity, decreased intellectual ability, poor judgment, etc. "Hunger brings into the open the pre-existing neurotic and psychopathic tendencies," says Sorokin,[47] and de Castro[12] mentions as "dissolvant effects of starvation on the human personality banditry, morbid mysticism and moral depravity, etc."

In the state of chronic starvation, men may try to subdue the pain of hunger in other ways than by acquiring more food. I shall describe the dangerous habit of coca chewing as a method for the inhibition of the chronic hunger sensation of an undernourished population.

It is the privilege of the physiology of nutrition to find the scientific basis for those national economic decisions which can raise the quantity and quality of the necessary food. The dangerous effects of starvation in contributing to personality deterioration, together with the additional dangers of addiction, might be abolished, and a problem that is mainly psychological might thus be solved by better nutrition.

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The discussion of coca chewing will lead us to the problem of its origin and also of its remedy. It will give us an occasion to look for other possible ways of fighting hunger and to discuss the physiological mechanisms which cause the feeling of hunger.

Coca Chewing in South America

In several regions of South America, such as Peru and Bolivia, in some smaller regions of Colombia, formerly also in Ecuador, and occasionally in mining regions in Argentina, the leaves of *Erythroxylon coca* are chewed by several millions of people. One might rightly say that this food habit is one of the most discussed social problems in some of these countries, especially in Peru and Bolivia. It has very serious economic aspects and has often been fought with energy. The question has produced an enormous amount of discussion in popular and medical journals during the last fifty years. It is fortunate that the discussion of coca has finally reached a scientific basis, but it is still a matter causing much social and political unrest. It is of special interest to the physiology of nutrition, because it gives a unique demonstration of how complete nutrition may be the clue to the problem of eliminating a dangerous and deleterious habit of a whole population.

Erythroxylon coca is a small shrub, which is cultivated in large and carefully kept plantations in the eastern and northern valleys of the Andes under 2000 m altitude. In Bolivia, the largest plantations are in the Yungas. The leaves contain about 0.5 per cent cocaine,

calculated for the sun-dried material. The plantations are owned by rich families of Spanish origin, but there are also small land owners, Indian aborigines or mestizos, who plant a few shrubs for their own use and for small business. The leaves are plucked several times, up to five or even six times a year. This makes coca a continuously paying plant. The leaves are dried on stone floors by the tropical sun, quickly packed and either on mules, or more often on lorries, sent to the Altiplano. Here the larger part of the population lives at an altitude of 3500 to 4000 m under hard climatic conditions, and here coca is consumed. Only a few roads lead over the passes of the Andes, and on these the coca tax offices are situated, which make a rich revenue from this trade.

In 1948 the United Nations Commission on the Coca Leaf was sent to Peru and Bolivia on request of their governments to study the problems of coca chewing. As a member of this commission, I had occasion to study especially the medical-biological side of the problem. I shall refer mainly to our extensive report, which collected and worked up critically all the available data. It was published as E 1666/1950, Publication of U.N. in May 1950.[56]

The statistics on the *production* of dried coca leaves are inexact in certain respects. Obviously, only coca produced on big farms is registered for taxation and statistics. Thus all data have to be taken with some criticism. The production of dried coca leaves for the last available year was 6,500,000 kg in Peru and 4,000,000 kg in Bolivia. These have a value of 357,500,000 soles in Peru and 220,000,000 bolivianos in Bolivia. The coca tax in Peru in 1950 was 3,758,400 soles, i.e., 0.23 per cent of the national income. In Bolivia, the tax is taken in each department separately. In the two main producing departments of La Paz and of Cochabamba, the coca tax between 1942–48 was 79 to 90 per cent of the whole state income. It is characteristic for this situation that the flourishing University of La Paz was financed by 15 per cent of the coca tax. In 1947, Peru registered 311,123 kg for export. In Bolivia, the export to the miners of Argentina and Chile was uncontrolled. The amended world total need of cocaine for 1953 (Estimated world requirements of narcotic drugs in 1953, U.N. E/DSB 10/Add. 3., p. 16) could be produced from about 9000 kg coca leaves. About 300,000 kg are exported from Peru for flavoring substances. In these the alkaloid is destroyed before it is used in American soft drinks.

Even after subtraction of these quantities, there remains a statistically definite quantity of 10,000,000 kg coca leaves with 0.5 per cent cocaine, i.e., a cocaine content of 50,000 kg. This is the quantity which is consumed by the chewers in Peru and Bolivia. If we allow that only about 70 per cent might be absorbed, the quantity of cocaine absorbed is reduced to 35,000 kg.

We may now calculate the number of *coca leaf chewers,* the *coqueos,* and then try to estimate the quantity of cocaine which the average person is consuming.

Coca leaves are mainly chewed by the Indians, but also by many mestizos. The white population generally does not chew, although exceptions are known. The region in which it is chewed is the Altiplano, where, at altitudes of 3500 to 4000 m, on the plateau between the two Andean Cordilleras, the greater part of the indigenous population lives. It is a cold climate; trees are scarce; and the temperature is between +3 and +25° C.

There are two different Indian tribes here: The *Quechua* are open-hearted, extroverted people, whose livelihood comes from agriculture and who keep llamas and a few cattle, plant chinua, beans, and several different sorts of potatoes. Their number is about 2,500,000 in Peru and 1,165,000 in Bolivia. The other tribe is the *Aymara,* who speak a different language and are said to be more introverted. They are mixed among the others. A large group occupies the region around Lake Titicaca. There are 315,000 in Peru and 862,840 in Bolivia. They, too, are predominantly agricultural. For comparison, I might mention that the total population of Peru is 6,340,799, and of Bolivia 3,922,000, according to the 1949 census.

Thus, from about ten million inhabitants of the two countries, more than half are pure Indians, and about one-quarter or more are mestizos. Cuzco, the ancient Inca town, is the spiritual center of the Indian population, where some years ago the Pan-Indian Congress was held.

Of course, not every Indian and mestizo is a chewer, or coqueo. Boys start at about 14 years of age and are proud to show that they are grown up. Women chew more seldom, perhaps about one-fifth of them. Thus, since the number of children below 14 in Peru is 21.4 per cent, one might say that at least 2,500,000 people are regular coca chewers, not counting the mestizos, who must increase this number at least to three million. It is mainly the heavily working population, agricultural peasants and miners—the proletariat, which chews.

The leaves are bought on the market, and a handful, about 25 g, is taken at a time. They are kept in a special bag, carried about the waist. If one relates the quantity of coca leaves produced to the probable number of chewers, 10,000,000 divided by 3,000,000 = 3.3 kg per year, and 9 g per day are consumed. This is certainly too small a figure and shows how uncertain the statistical data are. The usual daily dose, according to the experiences of several observers, is more like 25–50 g. Inveterate chewers take 100–125 g, and real addicts even 200 g per day, i.e., 1 g of cocaine per day. But the latter are very rarely found. There is not a continuous increase of the dose of coca leaves, nor is there an uninhibited drive to obtain the drug. The leaves are taken carefully, almost with ceremony. One by one they are pushed into the mouth, and then from a special little flask, made from a gourd, *lipta* is taken; this is a strong alkali mixed with water; with a spoon of horn the lipta is pushed between the leaves in the mouth. The bolus of leaves is kept on one side of the mouth and mixed with saliva. The cheek shows a characteristic bulge. This is so characteristic that one recognizes the chewer in the street. Chewing goes on for two to two and a half hours. After that a new dose will be taken. The alkali of the lipta helps to destroy the walls of the leaf cells. A more important role may be that it makes the extract of the leaves alkaline and this is swallowed with the saliva, neutralizing the hydrochloric acid of the stomach. The alkaloid is thus not hydrolyzed and will therefore be absorbed in its active state as alkaloid salt.

In the huge tin mines of Catavi in Bolivia, official shops owned by the Patino Mining Company sell to the miners packages containing 125 g of coca leaves. They generally buy two for one week. It costs about 25 per cent of their weekly income. It may be remarked, however, that they do not spend money on tobacco smoking, which is quite unusual with the Indians. Not long ago it was customary to pay the Indian partly with coca leaves, but this seems now to have been given up.

I spent three and a half months, from September until the middle of December 1949, in many different parts of these countries, in the regions of the chewers and in the regions of the producers. We crossed these areas by car, by train, by airplane, and occasionally on horseback, traveling many thousand kilometers. We visited, on the Altiplano, Puno, Cuzco, La Paz, Catavi, Cerro de Pasco, and Morococha, where the main coca consumers live. We also visited the regions of production: the Yungas of Bolivia, Coroico, where bananas grow up to 2500 m, Cochabamba with its university, Quillabamba, where the big forest of central South America begins, Tingo Maria on the Rio Urabamba, and then Cayamarca and Trujillo in the northern parts of Peru.

Our working method was to arrange round-table conferences with the help of the Central Government and the local authorities in all these places. At these, all the local groups of the producers and the consumers, the latter represented by miners, factory workers, lorry drivers, etc., were present. Also the local Roman Catholic clergy, the Protestant missionaries, judges, doctors, and military authorities were always present. They gave their opinions very open-heartedly. About twenty-four such conferences were held. We heard the story about this miraculous and sacred plant of the Incas, about its marvelous action in providing unknown energy to men. And then, the

condemnation of its race-ruining action, as it is often called. We heard some doctors say that coca has to be condemned, while other doctors thought that the role of coca is overemphasized and that it is not the explanation of the present low status of the Indian population. There is the opinion that one cannot live and work in these altitudes without coca, or the similar earnestly propagated view of scientifically trained colleagues, that the Indian race does not react in a deleterious way to cocaine, and that coca might even be of food value. On the other hand, there were the opinions of the working class leaders, the syndicalists, who prayed that coca should be taken away from their people. They were convinced that it destroys character and inhibits progress. It became very clear that this centuries-old custom, surrounded with the old mystery of the miracle that it makes man forget hunger, tiredness, and sorrows, is so deeply rooted that it is hard to find the truth. The fight about coca goes on in pamphlets and in the daily press; often it is the fight between capitalistic landowners and syndicalist workers' unions; or between a government party and its opposition. However, the deep conviction of the harmfulness, as explained by workers, clergy, and military, as well as the generous agreement of the representatives of the planters to help to replace coca by changing their plantation policy, left a deep impression on me of the high spirit of these two classes of the population in these countries.

The Cause of Coca Chewing

The chewing of coca leaves was certainly known in the times of the Incas, i.e., before 1532, the date of the Spanish occupation. It seems that after the Spanish conquistadores destroyed the Inca Regime, the coca leaf, which had until then been a privilege of the governing aristocracy, became available to the working class. Perhaps it was one of the ways to convince the people that Spanish rule meant a liberation from Inca tyranny. Coca cultivation and chewing spread and became general during the last 400 years.

The action of cocaine has been thoroughly studied. On the basis of these studies, the opinion of those who defend coca chewing may be criticized and the cause of this habit might be found.

The alkaloid cocaine which is contained in these leaves in a concentration of 0.5 to 0.6 per cent is the oldest known local anesthetic. I refer to the textbooks of pharmacology of Poulson,[43] Möller,[40] and Eichholtz,[14] and to the monographs of Mayer (1926), Merzbacher (1929),[39] and others.

Cocaine has an inhibitory action on peripheral nerves and on sensory nerve endings. It also has a specific action on the central nervous system, even in very low concentration. It has been much used for its local anesthetic action and is still preferred for this purpose for the cornea of the eye, on mucous membranes, and on serous membranes. It abolishes the sensation of pain and also of taste and smell. An anesthesia of the stomach with cocaine takes away the sickish feeling in stomach disease. Mucous membranes after cocaine also show a vasoconstriction. Stomach secretion is stopped. According to Möller, the different actions of cocaine are complicated and not definitely explained; apparently not only sensory nerve endings are inhibited.

This anesthetic action depends on the presence of the free alkaloid base. At a very acid reaction, as in the gastric juice, a dissociation of the alkaloid takes place and the action is destroyed. Only in alkaline solution is the alkaloid stable and active and absorbed in the active state. Obviously, this must be one of the reasons why the coqueo mixes a strong alkaline substance (lipta) with the leaves.

The action on the central nervous system is what is desired by the addicts, the cocainists. Möller calls the condition resulting from cocaine an "euphomania" and thinks that cocaine is more euphorically active than morphine, heroin, amphetamine, benzedrine, or alcohol. Eichholtz describes the central cocaine action and also the result of coca leaf chewing by saying: "It satisfies the starving, gives new strength to the tired and exhausted, and makes the unfortunate forget his unhappiness."

From these central activities the inhibition of the feeling of tiredness was thoroughly

studied as early as 1891 by Mosso.[41] In experiments with the ergograph he confirmed this action and showed that it does not occur if one stimulates the muscle directly or indirectly, but is seen with voluntary muscle work. Fifty to 100 mg of cocaine had such an action. Thus the drug certainly has central nervous system activity. For such reasons cocaine was often used for "doping."[44]

It is equally certain that cocaine stops the painful sensation of hunger. The frequently quoted story of the Indians who walk for days without food and rest over enormous distances may be true.

The actual working capacity of a coca chewer is not increased, as has lately been proved by Zapata-Ortiz with experimental evidence.[61] This has also been confirmed by different engineers in the mines. Their opinion was that coca chewing is useful for the Indian to keep him steadily working, but his total output of work is always less than that of non-chewing European miners. The favorable action against tiredness is not a mysterious capacity to increase the energy production, but is the result of a central nervous inhibition of the sensation of tiredness. Similarly, the capacity of coca to stop the feeling of hunger is not the result of some nutrient value of the coca leaves as Cardenas has recently pointed out in a well-written article.[9] It is the result of central inhibition of hunger feeling.

The thesis that coca chewing is necessary for life in high altitudes is contradicted by the fact that there are also quite a number of chewers in the lowlands; we found them even on the Guano-Island, San Lorenzo, or in the coca plantations of Quillabamba.

On the other hand, in the Altiplano there are many hard-working people who do not chew. The best examples are the over 100,000 whites of La Paz, a town with 300,000 inhabitants at 4000 m altitude. It is built like a Central European town, with tram and motor car traffic, with a skyscraper university, and a well-adapted white population, which does not chew coca. Similarly, none of the other much-quoted signs of a changed pharmacological excitability of the Altiplano population, nor changes in fertility, could be verified. It was not difficult to collect these negative proofs. They are extensively discussed in our report.

It is hardly necessary to prove that coca chewing has deleterious effects on the whole personality of the chewer. We have defined it as a dangerous habit, which might become an addiction, and should therefore be prevented. Unfortunately, the "WHO Expert Committee on Drugs liable to produce Addiction" had given a far too strict definition of habit and addiction. Our actual observations did not meet their definition of addiction. After much discussion, this committee decided in its meeting from June 22–27, 1953 (*Bulletin on Narcotics* 6: 47, 1953): "The term 'cocainism' is applicable to the taking of the pure alkaloid for non-medical purposes. Coca chewing, 'cocaism,' must be considered a form of 'cocainism.'"

Even the enemies of coca in Peru are against calling their whole Indian population "toxicomaniacs," i.e., addicts. But whatever difficulties arise in such definitions, it is certain that coca chewing should be abolished, since it is a chronic intoxication with cocaine, with all its deleterious mental consequences.

As mentioned above, we do not believe that coca is chewed because it is necessary for life at high altitudes, nor do we believe that it has value as a foodstuff, nor that it contains some substance, identical with cocaine or not, which might enable a person to increase his output of muscular energy. None of these is the cause of this very general habit.

We came to hold the conviction that the chewing of coca leaves is a method for inhibiting the feeling of hunger in a chronically underfed population. This opinion is identical with that of those writers who have studied coca chewers most thoroughly, as did the pharmacologist of Lima, San Marcos University, Prof. Guttierez-Noriega,[18] and the hygienists, Prof. Paz-Soldán[42] and Dr. Kuczynski,[25] and others. The habit is, however, so much surrounded by mysticism that it is difficult to explain it on a biological basis. No doubt there are many who use this habit without being in the condition for which it was originally used.

All writers agree that "the food of the Indians throughout the South American continent is defective and utterly inadequate to compensate for the energy used up by their daily work," as V. G. Garces[15] wrote. Guttierez-Noriega[18] mentions "malnutrition as the main cause of the misery of the indigenous population and the cause of coca leaf chewing."

The nutrition of the Indians of the Altiplano is mainly based on Quinua (*Chenopodium quinua*). The total annual consumption per head is 53 kg in Puno and 10 kg in Cuzco. Its nutrient value, based upon rat experiments, is said to be as good as that of casein. A similar high value is ascribed to Canihua. The amino acid contents have been analyzed and are said to be of the order of a complete protein.

Other foods are potatoes and beans. Very little meat and no milk products are eaten. No fruit or vegetables are available on the Altiplano, but lately bananas have been brought up on lorries and sold at the market.

The observations which might lead to an analysis of the cause of coca chewing are the following:

Higher paid Indian workers do not chew. Thus the workers of a textile plant in Cuzco, who worked on modern machines, were all non-chewers. In the laboratories of a metal manufactory, we found many Indian laboratory assistants who were all non-chewers. Even in the mines, where it is said that every miner is a chewer, those Indians who were at the most responsible posts, at the mine lifts, did not chew. Nor did the leading miner who handled the electric drill and blasted the ore with dynamite. He was highly paid and did not chew coca. No chewer would be accepted for such a post. Of course one might ask whether non-chewing is the expression of a primarily higher cultural status which makes the person adapted for the higher quality work. However, the impression we got was that they do not chew *because* they are well paid and better fed.

In contrast to this, the ordinary miners whom we visited deep under the earth, hammering the ore out of the rock under very difficult conditions, are all chewers, and if questioned as to why they chewed, they answered: "Because then I am not hungry and tired and not afraid."

Another group of non-chewing Indians are the 4000–5000 Protestants around Puno, on Lake Titicaca. They have been taught by North American missionaries not to chew. They have better and cleaner houses; they eat better and are generally on a higher social scale than the other Indians of the region who are all coqueos. One might say that they are better fed because they can use for food the 25 per cent of their income which they have saved on coca. It is difficult to prove that the better nutrition keeps them from coca chewing, because it might be that the primary spiritual influence which acted on them had the secondary consequence of better nutrition.

Of decisive value is the information given us from the leading military doctor of Bolivia and by several other military doctors in Peru: Practically all recruits of Indian or mestizo origin are coqueos. When they enter the military service, they find that in the barracks and in service in general, coca chewing is prohibited. But from the very first day, they are well fed and receive much more meat than they were used to. They stop chewing, and even outside the barracks do not try to get coca leaves. They simply forget about it, as the doctors explained. Neither signs of abstinency, nor of a craving for the drug can be observed. After a few days the habit is given up, and consequently the efficiency of these men is much increased. The general explanation is that it is the result of the better nutrition. I have lately asked and again received confirmation of this observation from a Peruvian colleague.

A negative confirmation is also available. In the war of the Gran Chaco between Bolivia and Paraguay, the Bolivian army suffered terribly from insufficient food. Soldiers and officers were starving. It became necessary for the military authorities to allow coca chewing, and under these conditions not only the indigenous soldiers but also the white officers became chewers of coca leaves.

After we had cited these examples in our report, the following experiment was briefly

described by Dr. Vicente Zapata-Ortiz[61] of the Pharmacological Laboratory of the University of San Marcos, Lima. In Huancayo, on the Peruvian Altiplano, eight coqueos were kept under careful observation for one week. They received abundant food and "it was found that they spontaneously did not take Coca. Their nutritional status became obviously better and their weight increased. When they were asked whether they wished to continue Coca chewing and to have poor food, or stop chewing and eat well, they all decided for the latter."

Thus we do not hesitate to conclude that with an improvement of the nutrition of the indigenous population, the dangerous habit (or addiction) of cocaism will disappear.

This means, of course, that the coca growers will have to change their production. There is the possibility of planting coffee, tea, maize, etc. on the same site. Such a transformation of the economy of these countries has important financial consequences. It might need 15–25 years till the coca planters can transform their production. Meantime, the social conditions of the Altiplano population would become better, and a trial should be made to equalize their nutrition with that of the military.

At present, an agreement has been reached between the Peruvian government and the Economic and Social Council of the United Nations to perform a large-scale social experiment in some region where coca is not produced but is generally chewed. It is believed that with this better nutrition the population will give up chewing spontaneously, as they are able to do in the military service.

The Physiology of Hunger

To reach an understanding of the observation that cocaism can be stopped by complete nutrition, it is necessary to discuss the physiology of hunger.

The explanation of hunger has gone through great changes during the last half-century. In the 19th century (see Mayer), Magendie, Tidewald, Milne-Edwards, Roux and Michael Foster believed that hunger is a general sensation of central origin. But in 1912 Cannon[6] pointed out that hunger seems to be caused by peripheral stimuli, the feeling of emptiness in the stomach. Therefore, filling, even with indigestible material, can inhibit this sensation, of course only transiently.[1] This "peripheral hunger theory" was enlarged by Carlson,[10] who showed that if the stomach is empty for some time, intensive peristaltic movements begin, which are connected with the unpleasant feeling of hunger. He also showed that these stomach movements can be inhibited by chewing an indifferent substance (gum). Carlson also mentioned smoking as an inhibitor; this will be discussed later. He also said that hypoglycemia might be a cause of the stomach movements which give the feeling of hunger.

The opinion that hunger is caused by peripheral stimuli of emptiness or stomach movements could not be maintained after surgeons had successfully removed the whole stomach and hunger was still felt by these patients. Also, Grossman and Stein[17] showed that total denervation of the stomach does not abolish hunger sensation. Thus the gastric hunger contractions and the associated epigastric pang are only one, and a dispensable, component of the entire complex of hunger sensations.

After 1922 it became known that insulin-hypoglycemia is accompanied by a strong sensation of hunger and tiredness. It was observed that this could be stopped immediately by a few pieces of ordinary sugar or, better, glucose. One observation was made in the *British Medical Journal*, that a remedy for elderly golf players after the eighth hole is to eat two pieces of sugar. Thus the opinion became widespread that hunger is caused by hypoglycemia.

This was much discussed in connection with the action of nicotine or smoking, which was recognized as a method of inhibiting hunger by causing a hyperglycemia. Some explained this according to the knowledge of their time as a nicotine inhibition of stomach movement, and L. R. Müller explained it as an action of nicotine on the central nervous system, from where it inhibited the stomach movements. Several authors showed that smoking of ciga-

rettes produces hyperglycemia and explained according to the hypoglycemic theory of hunger that, therefore, smoking inhibits hunger.

Caponetto,[8] Lundberg (1931), Töppner,[49] Greenberg and Haggard,[16] and Lehmann and Michaelis[26] explained that nicotine stimulates epinephrine secretion and this causes the hyperglycemia. Wacholder[58] has observed the hyperglycemia after the smoking of one cigarette in 19 persons. He found: "a far reaching parallelism between the . . . changes of hunger-feeling and the estimated blood sugar values . . . a disappearance of hunger with increase of blood sugar . . . etc." But the increase of blood sugar lasts only 60–80 minutes. A second cigarette, or more nicotine, leads to a reversal, a hypoglycemia, and a nutritional hypoglycemia may be prolonged.

Thus the smoking of cigarettes has certainly some immediate effect, but it never stops hunger for long. However, in 1949, Janowitz and Grossman[23] found: "that fluctuations in blood sugar levels in fasting subjects were in no way related to hunger sensations . . . intravenous glucose did not stop hunger sensations." After an injection of insulin, hunger was felt at a blood sugar level of 42 mg per cent and disappeared at 61 mg per cent. In contrast to this, after a long period of fasting in humans, the hunger sensation appeared at the 71–89 per cent blood sugar level and after feeding disappeared if the blood sugar level was only 4–9 mg per cent more. Similar results were obtained by Scott (1938). On the basis of such experiences, the blood sugar theory of hunger was lately transformed by Jean Mayer[32–34] into the theory of the "glucostatic mechanism of regulation of food intake." He points out that the hypothalamic centers contain chemoreceptors for blood glucose (glucoreceptors). Intravenous injection of glucose, fructose, or epinephrine decreases the food intake of rats. Insulin hypoglycemia, on the contrary, increases their food intake, even with glucose at relatively high levels. This, however, was contradicted by others (McIntyre and Burke[38]). Sucrose and fat have no influence on food intake. There is, however, no constant relationship between blood glucose, appetite, or the sensation of hunger.

One serious contradiction seems to be that the high blood glucose level of diabetics does not destroy appetite, which is rather high in these patients. Another interesting fact is that gold-thioglucose produces enormous obesity in mice and rats (Brecher and Waxler[4]), perhaps by blocking some enzyme systems concerned with the sensitivity to glucose. Mayer thinks that it is the "utilization of glucose" which is the stimulus for hunger. He discusses the phosphorylation of glucose as the decisive factor, and he writes: "It is possible that the passage of potassium ions into the glucoreceptor cells along with the glucose-phosphate level is translated into a neural mechanism.[33]" He therefore determined peripheral arteriovenous blood glucose differences, as an index of the rate of glucose utilization. These values were found to correlate closely with the calorie intake of the subject and with the hunger sensation. If this difference was more than 10 mg per cent, no hunger was felt. At differences of 0 or somewhat more, hunger was always present.

The Hunger Center

In recent years, most valuable experimental results have been collected which prove that the hypothalamus contains centers for the regulation of food intake. Ranson, Fisher, and Ingram[44] and Hetherington and Ranson[19] showed that the destruction of parts of the medioventral nuclei of the hypothalamus leads to an enormous increase of food intake and consequently to obesity in rats. Anand and Brobeck[3] found that more lateral lesions cause anorexia. This region of the hypothalamus thus regulates food intake and its stimulation leads to a feeling of hunger. It has been questioned how far hunger is identical with the drive for food intake. It has been pointed out that the drive for food intake, or appetite for food, is not caused by hunger-stimulus. We might point out how continuous the regular food intake of certain birds is, or how feeding goes in certain periods in other animals. Food intake seems rather to be a rhythmic activity in the life of the

animal and not caused by the painful stimulus of hunger.

I doubt whether such a differentiation is right. The relation of the drive for food intake (appetite) to the sensation of hunger has its analogies in the function of other autonomous centers: the respiratory center gives rhythmic stimuli to the respiratory muscles without waiting each time for an asphyxia as stimulus. Asphyxia is a general sensation analogous to hunger.

The heat regulatory center also has its daily rhythm. It keeps heat production and heat release in a constant equilibrium without waiting for a decrease of the body temperature. It is stimulated by peripheral stimuli of cold or warm receptors and only excessive stimuli lead to the unpleasant sensation of freezing or over-heating.

Similarly, the center for the drive for food intake keeps up a rhythm in the animal's life without the excessive stimulus of hunger. Thus hunger is a parallel to asphyxia or freezing in the function of the other hypothalamic (brain stem) centers.

Another common point in the function of these centers is their great influence on psychic behavior, through a strong general feeling or emotion of unpleasantness. On the other hand, psychic stimuli also influence the excitability of these centers, and can help to overcome, for some time, these general sensations.

It is also characteristic for these hypothalamic centers that in connection with the higher centers of consciousness they form habit-patterns. For example, the body temperature curve still continues for some days if the person changes his working time from day to night; but under the new conditions it will adapt itself slowly. Similar examples could be cited from the function of the respiratory center; or, again, an interruption of the regular rhythm of food intake, will lead to the sensation of hunger, and this will disappear if a new feeding rhythm becomes fixed.

Another characteristic of the autonomous centers of the hypothalamus and brain stem is that they are controlled by blood constituents (CO_2, O_2, blood glucose, blood temperature), but are also influenced by peripheral reflexes. The latter act long before the blood changes have occurred. Thus the respiratory center is influenced by the oxygen tension from the carotid sinus long before the lack of oxygen in the blood leads to a central stimulation. The heat-regulatory center of the hypothalamus is influenced by stimuli from the cold and warm receptors of the skin long before the blood temperature has changed, which will stimulate this center also.

Similarly, the hunger center, or, better, the centers of food intake regulation, do not wait until blood glucose is diminished, but are already stimulated by peripheral stimuli: by the emptiness of the stomach and by the intensive stomach movements. The maximal stimulation of a hunger sensation seems to be reached when the blood sugar decreases.

We may summarize as follows the characteristic features of these autonomic centers of hypothalamus and brain stem:

(1) Their stimulation leads to diffused sensations with a general character of pleasantness or unpleasantness.

(2) There is a strong influence on psychic reactions, and, conversely, psychic influences act strongly upon the excitability of these centers.

(3) They form fixed activity rhythms.

(4) They are primarily stimulated by peripheral stimuli.

(5) They are also stimulated, at a later stage, by blood constituents, and this leads to general sensations such as hunger, asphyxia, or cold.

The function of the hunger center agrees in all details with this scheme. Under physiological conditions, the drive for food intake and what we call appetite is not elicited by hunger sensation. A habitual rhythm and psychic influences lead to the food drive. Hunger is first evoked by peripheral stimuli like the emptiness of the stomach or the appearance of the stomach movements. These are early warnings like those of the skin receptors for heat regulation.

The rhythm of these hypothalamic centers is represented in the regular drive for feeding without a maximal stimulation by hunger.

If hunger appears, strong psychic reactions are evolved. It leads to restlessness, while satiety produces a pleasant, quietening psychic condition; and psychic influences can also adapt a person to continuous undernutrition by decreasing the stimulatory effect of the hunger sensation.

Finally, these hypothalamic and brain stem centers are influenced by drugs of a similar activity: barbiturates decrease the excitability of the respiratory center; procaine inhibits the heat-regulatory center and leads to a drop of body temperature; cocaine, as another local anesthetic, inhibits the hunger center and diminishes the drive for food.

Ways to Stop Hunger Sensation

There are different ways to stop the sensation of hunger. It is known from the description of the large hunger catastrophes in Russia and India that people were eating voluminous indigestible material, even earth, to stop the feeling of hunger (Cannon[6]). Curiously enough, the description of the condition of starving people during the last war gives no mention of similar cases. Neither Hottinger *et al.*[20] nor McCance and Widdowson,[37] describing starvation in concentration camps, mention this. Ancel Keys and co-workers,[24] in their famous "Minnesota experiment," noticed that the starving subjects took large quantities of tea or coffee, to fill their stomach, to diminish the feeling of hunger. They also took up chewing gum and cigarette smoking. Filling the stomach acts via peripheral sensible stimuli and also by inhibition of the stomach movements. Chewing is also a peripheral stimulus. Cigarette smoking, as mentioned above, has a more complicated inhibitory action through causing hyperglycemia. A third form of inhibition of hunger was chosen by the famous Succi, who was the object of Luciani's observations in Rome in the 1890's.[29] Luciani quotes Succi's own diary: He was drinking laudanum up to one-half ounce. This is 136.5 mg morphium. Later he took a mysterious liquor, which prohibited the stomach pain in the first days of fasting. This "famous liqueur" contained: laudanum, morphine, hashish, peppermint tincture, licorice-juice, and chloroform. He took 12 cc in two days. This mixture had certainly a central nervous inhibitory (narcotic) action.

The method used by the South American Indian to inhibit chronic hunger sensation by coca chewing belongs to the same group. It is true that the cocaine has first of all a local anesthetic activity and therefore inhibits the peripheral sensations of emptiness of the stomach, which leads to hunger feeling. It has been observed that in the coqueo the pharynx is completely anesthetized and no vomiting reflexes can be elicited. The anesthesia of the gastric mucosa will also stop the autonomic motor activity of the stomach and thus inhibit those primary peripheral sensations which are evolved by the hunger contractions. Coca chewing is, however, much more an action on the central nervous system, most probably on the hypothalamic hunger center. It eliminates the nervous sensations of hunger and the drive for food intake.

It should be mentioned that some synthetic preparations have similar actions: amphetamine and Pervitin. Amphetamine is used by some clinicians in therapeutic slimming cures as an inhibitor of appetite and food drive.

There seems to be an agreement that coca chewing has undesirable and dangerous general psychological effects also. It seems to us that with cocaine, the hunger-depressing concentration lies relatively far from the excitant concentration. Therefore, the coca chewer inhibits his chronic hunger and actually very seldom, only in cases of extremely large doses, reaches an excitatory state.

Comment

From the point of view of the nutritionist, it is satisfactory to see that adequate nutrition can abolish a dangerous habit, which becomes an addiction and doubtless has an unfavorable influence on the psychological state of the individual and the cultural evolution of a whole population. One might hope that this analysis of the biological background of a great social problem will lead to its abolition by means of better nutrition.

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Human Dietary Deficiency of Vitamin B_{12}*

By FRANK WOKES, PH.D.,† J. BADENOCH, M.D.,‡ AND H. M. SINCLAIR, M.D.§

EXPRESSED as percentage of the total dietary calories provided by the animal protein in the diet, the intakes of animal protein range widely in different countries; values vary from as high as 8 in parts of America down to less than 1 in India and

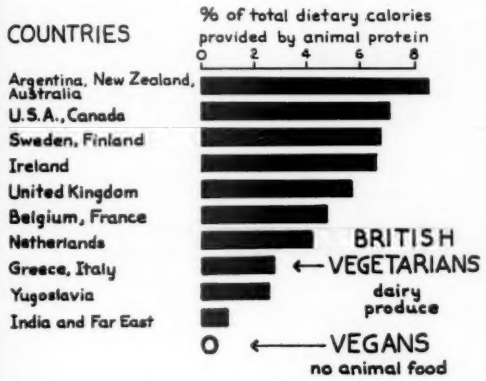

Fig. 1. Post-war animal protein intakes in different countries.

the Far East (Fig. 1). The high animal protein intakes in much of the Western world are indeed only possible because more than half the world's inhabitants are living on much lower intakes. Animal proteins on the whole are much more expensive than vegetable proteins; hence numerous workers have tried to replace animal protein by vegetable protein.[1,2] In Great Britain, during the last hundred years, there have been many thousands of vegetarians, who have refrained from eating flesh foods, though they still consume dairy produce. This has involved a reduction in their intake of animal protein to about half the normal British intake, without any visible ill effects. During the last ten years or so, some hundreds of these vegetarians have eliminated dairy produce from their diet, and called themselves *vegans*. After several years on this diet, which contains no animal protein, definite illnesses have gradually developed in some but not all of these vegans.[3,4] These illnesses are apparently due to a dietary deficiency of vitamin B_{12}.

Staple foods containing animal protein usually also contain vitamin B_{12}. On the other hand, this vitamin seems to be very scarce in vegetable foods, having so far been detected only in one or two, such as seaweeds[5] and ground nuts,[6] which are not consumed by British vegans. Their diets must therefore have been deficient in vitamin B_{12} and it is not surprising that their deficiency symptoms have been alleviated by administration of the vitamin.

Composition of Reported Vegan Groups

Before describing the occurrence of the deficiency symptoms in the group of British vegans, the composition of this group should be compared with that of a group of Dutch vegans recently studied at Leyden[7] and that of a smaller group of American vegans[8] (Table I). In both the Dutch and American vegans deficiency symptoms were much less in evi-

TABLE I

Composition of Groups of Vegans Studied

		Children	Adults
		0–18 years	19–83 years
American	males	2	14
	females	–	11
Dutch	males	–	39
	females	–	20
British	males	13	54
	females	19	63

* Presented at the Third International Congress of Nutrition, Amsterdam, The Netherlands (September 13–17, 1954) and published in *Voeding* (June 15, 1955).

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dence than among the British vegans. The British groups, however, contained more young people, who are probably more susceptible to vitamin B_{12} deficiency. The Dutch group contained four women and nine men aged 33 to 69 years who had been vegans for 2 to 15 years, 15 who had been vegans for 6 to 18 months, and 32 vegetarians who had lived on the vegan diet for short periods ranging from several months to a year.

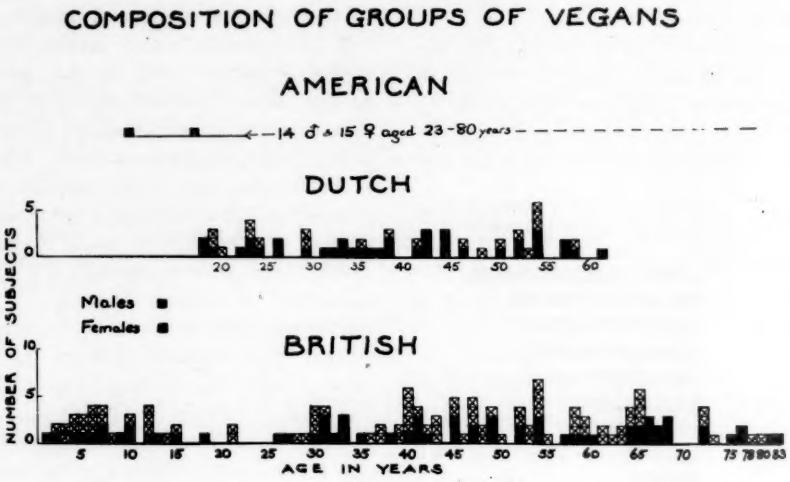

Figure 2.

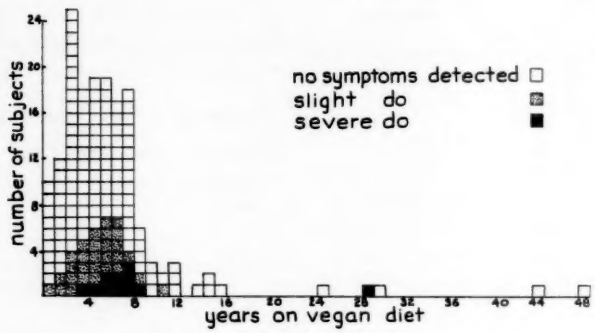

Fig. 3. Health in relation to period of veganism.

Health and Duration of Veganism

Another probable reason for the higher incidence of deficiency symptoms among the British vegans was their longer period of veganism. Even slight symptoms usually did not become prominent until they had been on the vegan diet for several years, and the more serious symptoms took still longer to develop (Fig. 3). Quite a number seemed to escape definite symptoms, even after more than five years on the diet. Children were rather more susceptible than adults, but some of them have continued to grow and develop satisfactorily for several years. The vegan mothers who have been more successful in rearing their children on the vegan diet often breast fed them for longer periods than usual.

Rate of Development of Deficiency Syndrome

The commonest and earliest symptoms have

been in the mouth; sore tongues were especially common. These *oral symptoms* generally cleared up after a month or two. *Paresthesia* was another fairly common symptom which developed more slowly and persisted rather longer. *Amenorrhea* and menstrual dis-

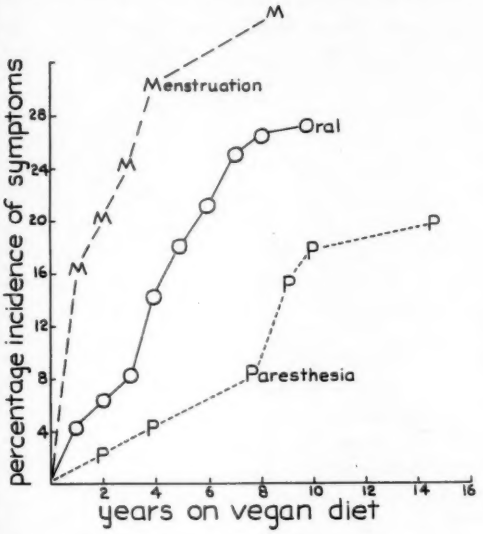

Fig. 4. Rate of development of different deficiency symptoms in vegans.

turbances were encountered in eight out of 22 women aged 15 to 45. They were also encountered among the Dutch vegans,[9] and are of interest in view of the fact that the main vitamin B_{12} deficiency symptoms observed in experimental animals are related to the reproductive processes (Fig. 4). *Nervous symptoms* were common among the vegans, but have not been plotted because their subjective nature makes their precise rate of development difficult to determine.

About 20 per cent of the subjects complained of pains in the back and spine. Some of these had stiff "poker" backs, so characteristic that they were termed "vegan" backs. Three deaths occurred, all among vegans of several years' standing. One of these was due to cancer which was present before the vegan diet was adopted. The other two deaths were associated with mental disease.

The percentage incidences of symptoms were lower in the Dutch than in the British vegans, e.g., oral symptoms—Dutch 12, British 27; paresthesia—Dutch 6, British 20. This was probably because 72 per cent of the Dutch vegans on whom these percentages were calculated had been on the vegan diet for less than two years, as compared with an average of five to six years for British vegans.

No clinical deficiency symptoms were observed among the 26 American vegans,[8] all of whom had maintained the animal protein-free diet for five years or more.

It has not yet been possible to arrange for some of the vegans with more serious symptoms to undergo detailed biochemical examination. The biochemical results which follow were obtained on vegans apparently in good health, most of whom had, however, experienced the less serious deficiency symptoms, especially those in the mouth, at some time during their period of veganism.

Serum Vitamin B_{12} Levels and Duration of Veganism

The serum vitamin B_{12} concentrations in the vegans so far examined have ranged from

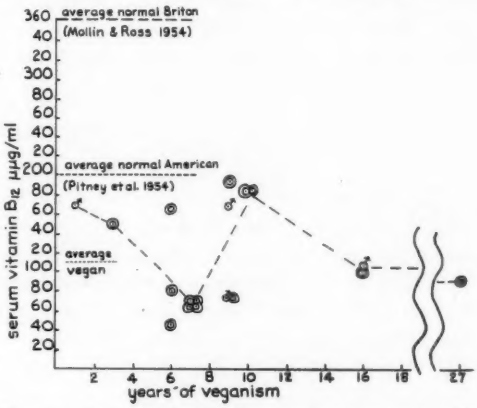

Fig. 5. Serum vitamin B_{12} levels compared with periods of veganism.

45 to 193 $\mu\mu$g/ml, and averaged 111 $\mu\mu$g/ml, as compared with the average normal values of from 200 to 320 $\mu\mu$g/ml obtained by several workers using the same method.[10,11] In more than half the vegans the average concentration was below 120 $\mu\mu$g/ml. In two of these it lay between 120 and 100 $\mu\mu$g/ml, suggestive of vitamin B_{12} deficiency.[12] In

the other nine it was below 100 $\mu\mu$g/ml, indicating definite deficiency, but this did not occur until six years on a vegan diet.

A curve drawn through the average vitamin B_{12} levels for each year of veganism for which data are available shows a steady fall during the first 7 years, followed by a recovery up to the original fairly high value between 7 and 10 years, then a more gradual descent (Fig. 5).

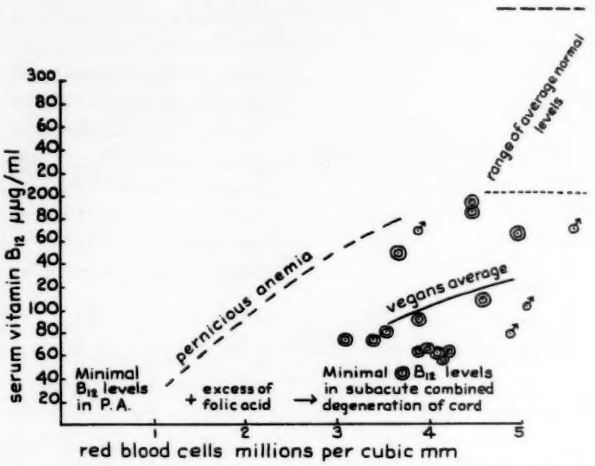
range of average normal levels
pernicious anemia
vegans average
Minimal B12 levels in P.A.
+ excess of folic acid
Minimal B12 levels in subacute combined degeneration of cord
serum vitamin B12 µµg/ml
red blood cells millions per cubic mm

Fig. 6. Serum vitamin B_{12} levels and red blood cell counts in vegans.

Serum Vitamin B_{12} Levels and Red Cell Counts

The red blood cell counts on some of the vegans indicated a slight anemia. However, correlation of the vitamin B_{12} concentrations with the red cell counts gave an average curve for the vegans which was quite distinct from the average curve in pernicious anemia, and was closer to the vitamin B_{12} levels associated with subacute combined degeneration of the cord (Fig. 6). This condition can be precipitated in vitamin B_{12} deficiency by administration of an excess of folic acid.[13] This might conceivably happen in vegans with low serum vitamin B_{12} levels who ingest large amounts of folic acid in vegetables. One of us[14] has previously described a vegan boy of 15 who developed dietary deficiency of vitamin B_{12} and suffered from classical subacute combined degeneration of the cord. This responded dramatically to treatment with vitamin B_{12} alone.

Serum Vitamin B_{12} Levels and Hemoglobin Levels

The hemoglobin levels in the vegans showed even less deviation from normal than had been shown by the red cell counts (Fig. 7). This might have been partly explained

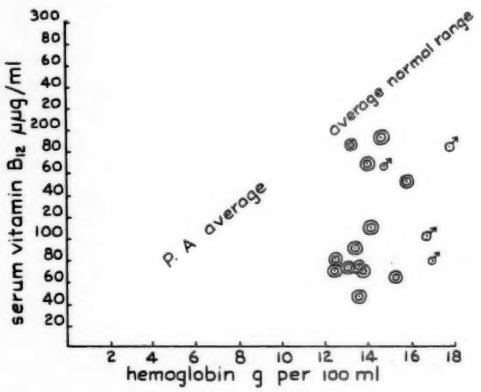

Fig. 7. Serum vitamin B_{12} levels and hemoglobin levels in vegans.

by an increase in the size of the red cells, such as was found by determination of their average diameters in the Dutch vegans. However, the mean corpuscular volume showed a

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definite increase above the normal value, comparable with the increase found in the Dutch vegans.

No correlation was established between the serum vitamin B_{12} levels and any of the other blood values measured, which included plasma Fe, platelet, leukocyte and reticulocyte counts, and erythrocyte sedimentation rate.

Tyrosyl Derivatives and Serum Vitamin B_{12} Levels

The deficiency symptoms observed in the nervous systems of some of the vegans might have been due to chronic toxicity caused by a keto-acid such as parahydroxy phenylpyruvic acid (PHPP), which is derived from tyrosine, and in the metabolism of which vitamin B_{12} may be implicated because of its relationship to thyroid activity (Fig. 8).

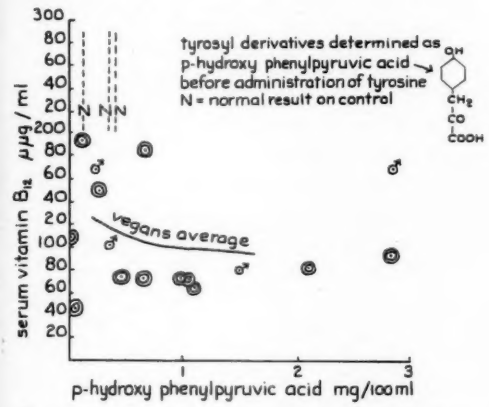
tyrosyl derivatives determined as p-hydroxy phenylpyruvic acid before administration of tyrosine
N = normal result on control
vegans average
serum vitamin B12 µµg/ml
p-hydroxy phenylpyruvic acid mg/100ml

Fig. 8. Serum vitamin B_{12} levels and tyrosyl derivatives in vegans.

The concentration of PHPP was therefore determined in the blood and in the urine of the vegans, and in controls on normal diets, before and after oral administration of tyrosine and glucose (54 mg and 1.8 g, respectively, per kg body weight). The initial concentrations, before administration of tyrosine, tended to be higher in the vegans with lower serum vitamin B_{12} levels.

The concentration of the tyrosyl derivative PHPP in the blood appeared to increase with the period of veganism up to the first 6 to 10 years, after which it may level out, but many more data will be needed to confirm these findings (Fig. 9).

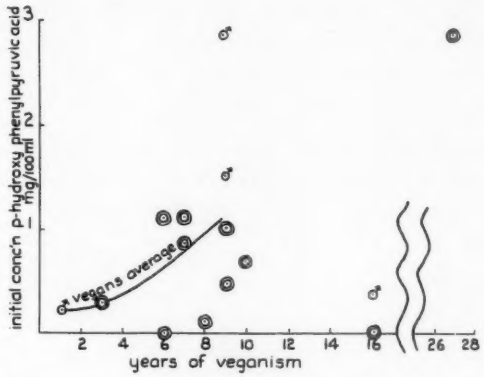

Fig. 9. Serum tyrosyl derivatives and period of veganism.

Cyanide and Thiocyanate Metabolism and Vitamin B_{12}

Another possible toxic agent is cyanide, which can cause demyelination in the central nervous system.[15] In the liver, cyanide is detoxicated by the enzyme rhodanese to thiocyanate, which is excreted in the urine. A measure of this detoxication is therefore provided by the urinary thiocyanate (Fig. 10).

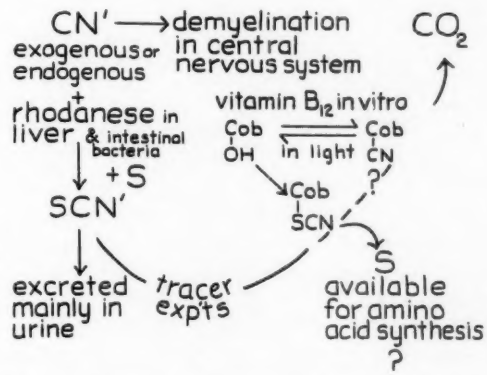

Fig. 10. Cyanide and thiocyanate metabolism and vitamin B_{12}.

Tracer studies[16] have shown that some cyanide is converted to carbon dioxide in the expired air. It may perhaps perform an essential function by acting as a "brake" on

biological oxidations. Vitamin B_{12} as hydroxocobalamin also can detoxicate cyanide, and serve as a carrier of cyanide and thiocyanate. Vitamin B_{12} has not yet been found in rhodanese, though closely associated with it in different organs and organisms. However, the urinary thiocyanate which is derived from cyanide may be associated with vitamin B_{12} in certain hypothetical systems which are discussed in detail elsewhere.[17]

The urinary excretion of thiocyanates may be increased by heavy smoking[18] or heavy consumption of beer or strong tea, or milk, eggs, and other sources of animal protein, all of which contain preformed thiocyanate.[4,19] None of these factors was significant in the vegans we have studied. Although cyanides and thiocyanates may be obtained from some raw vegetables such as cabbages,[20] which vegans sometimes eat, we did not detect significant amounts in samples of their diet, and therefore believe that the thiocyanate they excreted was mainly endogenous in origin.

Urinary Metabolic Data on Vegans and Non-vegans

The average daily excretion of thiocyanates per kg body weight was 135 μg among the vegans and 96 among the non-vegans, all being teetotal non-smokers. The vegan rate of excretion was 141 per cent of the non-vegan rate, but the difference was not significant, because complete 24-hour samples could not always be obtained to eliminate errors caused by the daily rhythm. This rhythm in thiocyanate excretion is, however, more or less parallel with the rhythm in total nitrogen excretion. By determining the thiocyanate/total nitrogen ratio, expressed in μg and mg, respectively, the standard error is much reduced, and complete collection of 24-hour samples is avoided.

The average thiocyanate/total nitrogen ratio in the vegans was 188 per cent of that in the non-vegans. However, part of this difference was due to the average total nitrogen excretion being lower in the vegans. When allowance was made for this, the corrected thiocyanate excretion in the vegans was still appreciably higher than in the non-vegans (Table II).

The average daily excretion of creatinine was significantly lower in the vegans. No creatine was detected. The Dutch vegans also had a lower creatinine excretion than the Dutch controls.

TABLE II

Urinary Metabolic Data on British Vegans and Non-vegans

	Average daily excretion (per kg body weight) of			
	SCN' μg	Total N_2 mg	Creatinine mg	Average SCN' ratio
Vegans*	135 ± 31	132 ± 15	17.3 ± 1.2	0.94 ± 0.05
Non-vegans*	96 ± 8	185 ± 27	27.1 ± 1.3	0.526 ± 0.04
Vegans as % of non-vegans	141	71	64	188

* All teetotal non-smokers.

SCN' ratio $= \frac{\text{SCN in } \mu\text{g}}{\text{total } N_2 \text{ in mg}}$ was determined on 181 vegan samples and 178 non-vegan samples.

Serum Vitamin B_{12} Levels and Thiocyanate Ratios

Turning to individual results, it has not yet been possible to establish a definite correlation between serum vitamin B_{12} levels and thiocyanate ratios in vegans, though there seems to be a tendency for lower vitamin B_{12} levels to be associated with higher thiocyanate ratios. Even after correction for the lower nitrogen excretions in the vegans, most of their thiocyanate ratios were above the normal range for teetotal non-smokers. Large doses (0.5 to 4 mg) of vitamin B_{12} can reduce the excretion of thiocyanates in both vegans and non-vegans (Fig. 11).

In one of the vegans the thiocyanate ratio increased from 1.02 before administration of tyrosine to 2.08 two hours later. In previous experiments without tyrosine, this vegan had shown an average ratio of 0.88; it seemed possible that administration of tyrosine might under certain conditions increase the excretion of thiocyanates. We have therefore carried out on each of the vegans who had received tyrosine a further experiment in which samples were taken of all the urine passed for at least 24 hours, no tyrosine being admin-

istered. The average thiocyanate ratio in each of these experiments was compared with the ratio obtained on the same vegan after tyrosine. The results indicated a stimulating effect of tyrosine on thiocyanate excretion in two of the other vegans. One of these had marked macrocytosis with serum vitamin B_{12} levels of 45 to 80 $\mu\mu$g/ml; this subject also had amenorrhea of several years standing and had experienced considerable loss of body weight. The other had slight macrocytosis with more normal serum vitamin B_{12} levels.

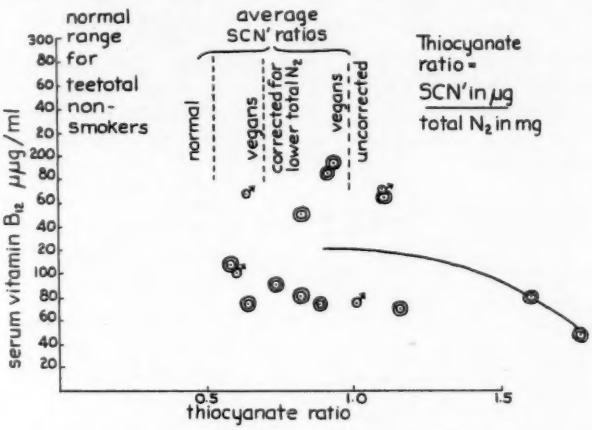

Fig. 11. Serum vitamin B_{12} levels and thiocyanate ratios in vegans.

Comparison of British, Dutch, and American Vegans

As this investigation is concerned primarily with vitamin B_{12}, we have not undertaken the detailed investigation of all the nutrients in the diets of all the vegans, nor endeavored to determine their status in regard to any vitamin except B_{12}. However, some data we have obtained on a few of the British vegans confirm the findings on the Dutch and American vegans, in that the vitamin and mineral intakes seemed satisfactory, except perhaps for calcium and vitamin D, and of course also for vitamin B_{12}. The dietary intake of this vitamin must have been extremely low, and it seems justifiable to assume that the differences in serum vitamin B_{12} values among the British vegans were due largely to differences in the supply of this vitamin from their intestinal flora.

The average body weight of the British adult male vegans was higher than that of the Dutch but lower than that of the American male vegans. In the adult females, body weight of British vegans was lower than that of the Dutch and American vegans. Data still being collected on the British vegan children indicate a subnormal rate of growth.

Unpublished data[21] obtained in a comprehensive study of the rate of growth and development of British vegetarian boys shows this not to be significantly different from that of non-vegetarian boys living under the same conditions. The vitamin B_{12} intakes of the vegetarian boys were no doubt considerably lower than the intakes of the non-vegetarian boys, but would be considerably higher than the vegan boys and girls.

The general blood picture in the British vegans did not differ significantly from that in the Dutch and American vegans. The only clear indication of dietary deficiency was provided by the serum vitamin B_{12} levels (Table III).

The results obtained in this investigation indicate the occurrence in man of a dietary deficiency of vitamin B_{12} similar to that encountered in animals also living on a diet free from animal protein. The poor rate of growth and development in animals on such a diet can be improved by addition of vitamin B_{12}, provided that their diet is not deficient in total protein.

TABLE III

Comparison of British, Dutch, and American Vegans

		British	Dutch	Amer.
% of dietary cal from protein		7*	9	10.4
Av. adult wt (kg)	♂	69.4	65.9	77.3
	♀	53.2	60.0	64.5
Red blood cells (m/cu mm)	♂	4.92	4.75	4.84
	♀	4.06	4.30	4.48
Hb %	♂	16.2	15.1	15.1
	♀	14.0	14.3	13.8
Serum vit. B_{12} $\mu\mu$g/ml		116	–	–

* Calculated by Worrall's method.[22]

The total protein intakes of the vegans must therefore be taken into consideration. This in the American vegans was 10.4 per cent, and in the Dutch long-term vegans about 9 per cent, on the calorie basis. In the British vegans it appears to have been about 7 per cent. This lower protein intake among the British vegans may indeed have been an important factor in causing the greater incidence of deficiency symptoms.

ACKNOWLEDGMENTS

We are grateful to Dr. D. L. Mollin for estimating vitamin B_{12}, to Miss Agnes Mayer for determination of keto-acids in the blood, to Mrs. B. Cane and Miss Janet Horsford for determination of urinary constituents, and to all the vegan and non-vegan volunteers who helped in this investigation.

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The Role of Vitamin B_{12} in Human Nutrition

By FRANK WOKES, PHD., F.R.I.C., AND C. W. PICARD, PH.D., F.R.I.C.

VITAMIN B_{12} first came into prominence for its therapeutic value in pernicious anemia. Soon afterwards, its effect in improving the biological value of vegetable proteins was established for different animal species, but there has been a much greater delay in obtaining conclusive proof of its "animal protein factor" activity in human nutrition, although a distinct growth-promoting effect has been observed with vitamin B_{12} when given as a dietary supplement to children in growth failure.[1] Other workers have not succeeded in demonstrating similar effects, but this may have been due to the tests having been made on children who, by virtue of their diet, could not be regarded as being markedly deficient in the vitamin.[2] However, human dietary deficiency of vitamin B_{12}, apparently *not* due to lack of intrinsic factor, has recently been described[3] among persons in Britain living entirely on vegetable foods and who are termed "vegans."[4] Consideration of the problems revealed by such human dietary deficiencies has led us to make the following speculations on the possible role of vitamin B_{12} in human nutrition.

COBALT AND CYANIDE

While there is still much to learn about the chemistry of vitamin B_{12}, one important point common to vitamin B_{12} and its analogues is the presence of cobalt in an organic co-ordination complex, by means of which cyanide can be taken up. Spectrophotometric studies on the effect of light on cyanocobalamin have revealed the existence of a system,

$$\text{cyanocobalamin} \underset{CN^-}{\overset{\text{light}}{\rightleftharpoons}} \text{hydroxocobalamin},^*$$

in which cyanide is readily taken up and liberated according to the experimental conditions, including pH and exposure to light.[5] Recent experiments *in vivo* have shown[6] that hydroxocobalamin can act as a remarkably efficient antidote in cyanide poisoning in mice, restoring life when death seems inevitable. Some of the vitamin B_{12} in the human liver is undoubtedly present as hydroxocobalamin, but even if the total liver store of vitamin B_{12} (apparently not less than 1000 μg[7]) were all hydroxocobalamin, the amount of cyanide with which it could combine would only be about 25 μg. Data on the cyanide content of certain foods,[8,9] as well as the doses of hydrocyanic acid which have been used in medicine, show that much larger quantities of cyanide may safely be ingested. The detoxication of these relatively large quantities is effected in the liver mainly by the enzyme rhodanese[10] (also known in America as transsulfurase[11]). A crystalline specimen of rhodanese has recently been prepared[12] and found to be colorless. Hence, it presumably does not contain cobalt, which in vitamin B_{12} takes up cyanide. Nevertheless, rhodanese and vitamin B_{12} occur together in many different organs and organisms, and their functions are probably interrelated.

The detoxication of cyanides and nitriles by rhodanese is effected by their conversion to thiocyanates, as can be readily demonstrated by experiments *in vitro* and *in vivo*. Administration of acetonitrile increased the thiocyanate concentration in the serum of mice[13] and the thiocyanate excretion in the urine of man.[14] Hence the urinary excretion of thiocyanates may serve as a measure of the cyanide-thiocyanate metabolism.

From the Ovaltine Research Laboratories, King's Langley, England.

* For the purposes of this discussion no distinction is made between hydroxo- and aquocobalamins. The term hydroxocobalamin is used to cover either form.

It has greatly interested us to find[3] in persons suffering from dietary vitamin B_{12} deficiency an increased urinary excretion of thiocyanate, becoming more marked when low serum levels of the vitamin indicated more severe vitamin B_{12} deficiency. This thiocyanate excretion can be diminished by administration of large doses of the vitamin. Since the thiocyanate excretion can be increased by smoking, by animal foods, such as milk and eggs, and also by the consumption of very large amounts of certain vegetables, especially cabbages, and of beverages, e.g. beer and tea, it is necessary to control intakes of these to obtain reliable results. However, in our experimental subjects, by the very nature of their diet and their attitude to stimulants of any kind, most of these potential sources of exogenous cyanide or thiocyanate may be ruled out. Analyses of the diets of such people in England have confirmed that the thiocyanate intake from such sources is negligible.[9]

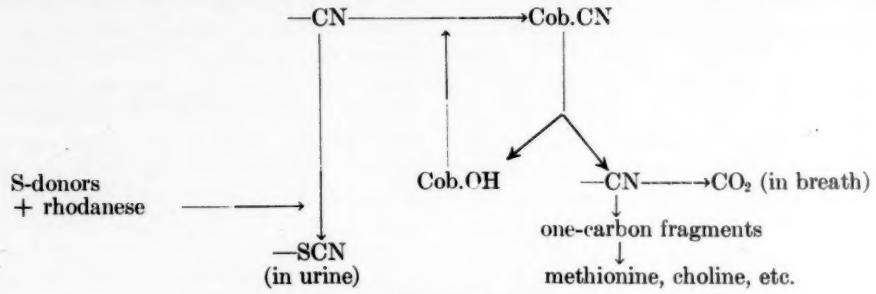

Fig. 1. Hypothetical scheme of cyanide utilization.

Cyanide Utilization Hypothesis

An attempt has been made by us to explain these findings in the first place by the following hypothesis summarized in Figure 1. In the liver, hydroxocobalamin ("Cob.OH") and rhodanese are assumed to compete for cyanide, and some will be taken up by hydroxocobalamin, converting it to cyanocobalamin ("Cob.CN"). The latter will carry out its various metabolic functions, one of which may be the supply of essential one-carbon fragments for the synthesis of labile methyl groups. Tracer experiments have shown[15] that the carbon in cyanide can contribute to a limited extent to the synthesis of methyl groups, and some goes out in the expired air as carbon dioxide. Finally, liberation of the cyanide from cyanocobalamin regenerates hydroxocobalamin, thus completing the cycle. The liver cyanide not taken up by hydroxocobalamin will be converted to thiocyanate by rhodanese and a sulfur donor which we think may be a sulfur-containing amino acid or a product of metabolism of such an amino acid. The thiocyanate is practically all excreted in the urine, very little being found in the feces.[16]

This suggested mechanism could explain the increased thiocyanate excretion observed in our experimental subjects who, because of their shortage of vitamin B_{12}, would not be able to utilize so much of the cyanide in the above cycle and would therefore have to excrete more cyanide as thiocyanate.

The hypothesis offers a twofold explanation of the methionine- or choline-sparing action of vitamin B_{12}: (a) The cobalamin cycle makes available cyanide radicals for the production of labile methyl groups or other one-carbon fragments, for example, for the conversion of homocysteine to methionine, a function of vitamin B_{12} now widely accepted[17]; and (b) in vitamin B_{12} deficiency, more cyanide would have to be detoxicated by the rhodanese system. Thus, there would be a greater need for sulfur donors, and these would not be available to fulfil other possibly essential functions. If methionine itself were the sulfur donor, any increase in detoxication by rhodanese would obviously diminish the methionine reserves of the liver. Evidence

that methionine can act as sulfur donor in the detoxication of CN-compounds by rhodanese *in vivo* has been furnished by studies[14] of the thiocyanate excretion of patients with liver disease. If, on the other hand, cystine or cysteine functioned as sulfur donor, this could likewise lead to methionine deficiency on the assumption that, in the presence of vitamin B_{12}, cysteine or cystine could act as precursors of methionine, as for instance in the system shown to exist in *Neurospora*[18] and certain other micro-organisms.[19] In this system, which is shown in Figure 2, the sulfur of cysteine is transferred to homoserine, forming cystathionine which undergoes cleavage to homocysteine, the precursor of methionine. There is a good deal of evidence[20] to suggest that this reversal of the normal metabolic pathway from methionine to cysteine, which is also shown in Figure 2, does not occur in higher animals. However, the biological possibility of such a pathway is demonstrated by *Neurospora,* and it remains to be shown whether, in the presence of vitamin B_{12}, it cannot occur also in higher animals and man.

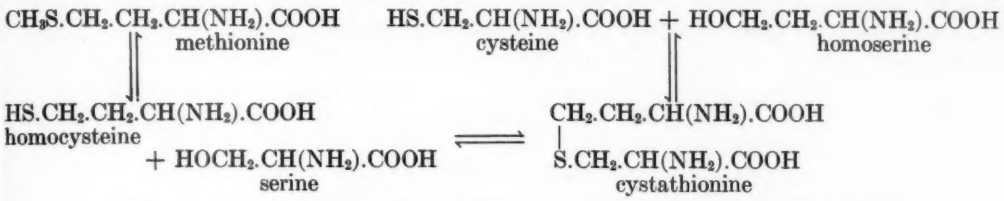

Fig. 2. Cystathionine mechanism of sulfur transfer between methionine and cysteine.

In view of these considerations, we think it would be of interest to look for evidence of methionine deficiency among persons having marked dietary vitamin B_{12} deficiency.

The hypothesis further takes account of the remarkable action of hydroxocobalamin as an antidote in cyanide poisoning and would fit in with the general view that vitamin B_{12}, as well as folic acid, is concerned with the metabolism of one-carbon fragments rather than with transmethylation.[17] It has recently been shown[21] that the carbon in cyanide and thiocyanate can contribute to the synthesis of methyl groups, probably *via* formate as an intermediate. The hypothesis accords with the finding, already noted, that in liver disease there is a lower urinary excretion of thiocyanate which is not increased by acetonitrile unless either methionine or cysteine also is administered.[14] Choline, on the other hand, does not effect the thiocyanate excretion under these conditions.

The suggestion that sulfur-containing amino acids, or their products of metabolism, may serve as sulfur donors in rhodanese action leads to a consideration of the quantitative aspects of this hypothetical cycle. The average thiocyanate excretion which we have observed in our vitamin B_{12}-deficient adults was of the order of 10 mg a day, compared with an average of about 6.7 mg observed in controls of similar body weight. These thiocyanate excretions correspond to 5.5 and 3.7 mg, respectively, of sulfur. The extra amount of thiocyanate sulfur excreted in vitamin B_{12} deficiency therefore appears to be too small to affect appreciably the reserves of sulfur-containing amino acids in the liver. However, *in vitro* experiments have shown[22] that the efficiency of these acids as sulfur donors in the rhodanese system is very low compared with that of sodium thiosulfate, but no proof has yet been forthcoming that, *in vivo,* naturally occurring substances other than these amino acids, or related substances such as glutathione, can serve as sulfur donors. In addition to the clinical findings[14] already noted that methionine or cysteine can act as sulfur donors, experiments on sheep have shown[23] that cystine, as well as thiosulfate, can act as sulfur donors in the detoxication of cyanide to thiocyanate. It may well be that the sulfur-containing amino acids do not act directly as sulfur donors, but only after oxidation to one of the intermediate stages in their metabolic breakdown to sulfite, sulfate, and thiosulfate. In any case, in view of the low

efficiency of the sulfur-containing acids, we think it possible that the rhodanese system in the body may require a large excess of these in order to function at an effective rate. It remains to be shown whether such an assumption is justified.

Another point to remember when considering this hypothesis is that cyanide has been shown[15] to contribute only a minor fraction of the required one-carbon metabolites, which again throws doubt on the quantitative validity of the hypothesis.

To sum up, the above hypothesis can be used to explain the observed facts on a qualitative basis, but encounters difficulties when the quantitative aspects are taken into account. The following alternative hypothesis, which is outlined in Figure 3, has therefore been considered.

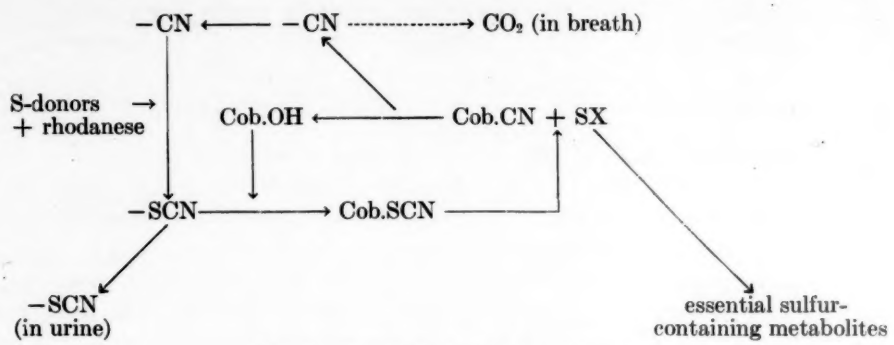

Fig. 3. Hypothetical sulfur transfer cycle.

Sulfur Transfer Hypothesis

Body tissues contain much more thiocyanate than cyanide.[15] From the evidence of *in vitro* experiments it seems possible that, in the presence of this large excess, hydroxocobalamin may take up thiocyanate to form thiocyanatocobalamin (Cob.SCN). This form of vitamin B_{12} has been found[24] to be as effective as cyano- and hydroxocobalamins in the treatment of pernicious anemia and in growth promotion of various micro-organisms used to measure vitamin B_{12} activity. It may therefore be argued that, under physiological conditions, thiocyanatocobalamin may revert either to cyano- or hydroxocobalamin.

For the purposes of this hypothesis, we have considered the possibility that thiocyanatocobalamin under physiological conditions reverts to cyanocobalamin, giving up its sulfur to an active intermediate, "X," to form "SX," which could be a precursor of biologically important sulfur-containing compounds, such as sulfur-containing amino acids, glutathione, or perhaps thioctic acid (also known as alpha-lipoic acid), a growth factor which appears to be essential for the oxidative decarboxylation of α-keto-acids in certain micro-organisms, including *E. coli*.[25] The cyanocobalamin will then complete the cycle by regenerating hydroxocobalamin, and the cyanide thus liberated will be reconverted by rhodanese to thiocyanate and again be available for the cycle.

If this mechanism operates in the formation of methionine, this might involve a reaction between homoserine and "SX" to give homocysteine which is then methylated to methionine. Such a mechanism would be analogous to the mechanism in *Neurospora* (Fig. 2), with "SX" taking the place of cysteine in supplying the sulfur to homoserine. Alternatively, if methionine were the sulfur donor in the proposed cycle, this mechanism demonstrates how vitamin B_{12} may catalyze the "normal" transsulfuration reaction, methionine→homocysteine→cystathionine→cysteine.

Discussion

It is not proposed at present to speculate on the nature of the active intermediate, "SX,"

or the mechanism of its formation. In such a speculation, the work on "active methionine" (S-adenosylmethionine),[26] which has been shown to yield homoserine on hydrolysis, is obviously of great interest. In any event, the cycle does suggest a way in which sulfur may be made available by vitamin B_{12} for the synthesis of sulfur-containing amino acids and related compounds. It has already been pointed out that cysteine or cystine could function as sulfur donors in the production of thiocyanate from cyanide by rhodanese. The cycle postulated here shows how, by means of vitamin B_{12}, sulfur might be transferred from these donors *via* thiocyanate to homocysteine and hence to methionine. Alternatively, if methionine were the donor, the cycle offers an explanation of the action of vitamin B_{12} in catalyzing the normal transsulfuration to cysteine, which is known to occur in higher animals.

In connection with the cyanide cycle which forms an essential part of our second hypothesis, it is of interest to note that the existence of a small but definite cyanide pool in equilibrium with the cyano-group of vitamin B_{12} has been demonstrated.[15] It has already been noted that some cyanide is metabolized to carbon dioxide in the breath. The expired air also contains traces of unaltered cyanide which, from recent observations,[27] appear to be proportional to the surface area of the subjects tested, indicating a direct connection with endogenous metabolism.

These various findings seem to us to provide support for the existence of some kind of cyanide cycle, like the one postulated here, in which minute quantities of cyanide, in conjunction with vitamin B_{12}, act as carriers of sulfur in relatively large amounts, as will be shown below. Indeed, it has been found[15] that the ratio of free cyanide to thiocyanate in body fluids is of the order of 1:1000. It is of interest to note that there is also evidence for the existence of a thiocyanate pool.

Sulfur Cycle

The proposed sulfur transfer cycle is not put forward as an alternative to mechanisms involving the synthesis and utilization of labile methyl groups and hence, among other things, the last stage of methionine synthesis, homocysteine→methionine. The evidence which has accumulated during the past few years strongly points to this being one of the functions of vitamin B_{12}.[17] Likewise, there is good evidence[28] that vitamin B_{12} is concerned in the conversion of glycine to serine which, as precursor of cysteine and, hence, glutathione, is clearly relevant to this discussion. In our view, however, a consideration of the cyanide-thiocyanate metabolism raises the question whether the vitamin may not play a vital role also in sulfur transfer in the synthetic processes outlined above.

Recent work has also shown that vitamin B_{12} is involved in the reduction of disulfide linkages to, and maintenance of, sulfhydryl groups, for example in homocysteine and glutathione, both *in vitro*[29] and *in vivo*,[30] and it may thus play an important part in growth promotion and regeneration of blood cells. However, this function does not account for the changes in cyanide-thiocyanate metabolism which formed the starting point for our considerations.

The quantitative aspect of the sulfur transfer hypothesis may now be considered. If the "animal protein factor" activity of vitamin B_{12} in man and other animals is concerned with the transfer of sulfur for the production of essential amino acids, as outlined above, then the amount of sulfur to be transferred has to be taken into account. Thus, for example, on the basis of a minimal daily adult requirement of 1 g of methionine, about 200 mg of sulfur would be needed for its synthesis. However, a study of the use of plant proteins in child feeding[31] has provided data showing that even in a diet in which all proteins are derived from vegetable sources, the average preformed methionine content may be expected to provide at least half of the requirements of this essential amino acid. Thus, in an adult on such a diet, the daily turnover of sulfur *via* vitamin B_{12} might have to be of the order of 100 mg. In a normal adult, the amount of vitamin B_{12} available for this purpose in the liver is taken to be about 1 mg. Calculations based on molecular weights show

that this amount of vitamin could carry about 25 μg of sulfur, which would have to be turned over about three times a minute to deal with 100 mg daily; this seems quite reasonable to expect in an enzyme system. The theory accords with the fact that the increase in thiocyanate excretion observed in vitamin B_{12}-deficient subjects is only relatively small, since it depends solely on the small balance of cyanide which, being unable to partake in the vitamin B_{12}-controlled cyanide cycle, is excreted as additional thiocyanate in the urine.

Role of the Liver

The quantitative considerations lead to the conclusion that, if this hypothetical cycle involving vitamin B_{12} does in fact exist, it almost certainly takes place in the liver, which should be carefully studied in attempts to test the hypothesis. This is based on two main assumptions, for which experimental evidence is required:

(a) the occurrence of thiocyanatocobalamin as an essential intermediate; and (b) the occurrence of the postulated intermediate, "SX."

Thiocyanatocobalamin, *in vitro,* appears to be less stable than cyanacobalamin and, *in vivo,* may be quite labile, as the hypothesis indeed suggests. This might explain why its presence in the liver and other tissues has not yet been reported, especially as its differentiation from cyanocobalamin by existing methods is not easy. Tracer experiments would seem to be indicated, both with micro-organisms and mammals, using for example thiocyanatocobalamin labeled with ^{35}S to see if the transfer of the sulfur to the amino acids or other sulfur-containing metabolites can be detected.

Valuable evidence about the possible occurrence and functions of both thiocyanatocobalamin and "SX" could also be provided by *in vitro* tests with both liver slices and suitable micro-organisms, supplied either with homoserine and thiocyanatocobalamin or with homoserine, thiocyanate ions, and hydroxocobalamin. In either instance, the substrate should be examined for the production of sulfur-containing amino acids. Labeling with ^{35}S should be of additional value.

Of particular interest would be a repetition of Armstrong and Binkley's feeding experiments with homoserine, choline, and cystine,[20] but with vitamin B_{12} added to the test diet.

We have tried to present our hypotheses in their simplest form, confining attention to the animal protein factor activity of vitamin B_{12}. We have therefore omitted reference to a number of factors which must eventually be considered to complete the complex picture of vitamin B_{12} metabolism. Among these factors are the relation of vitamin B_{12} to (a) folic acid and nucleic acid synthesis; (b) thyroid activity; (c) hemopoiesis and ascorbic acid. Consideration might also be given to the possible significance of cyanide and thiocyanate metabolism in red blood cells, especially in view of the recent evidence[32] for the presence of a porphyrin-like skeleton in the vitamin B_{12} molecule. The conversion of thiocyanate to cyanide by red blood cells has been demonstrated *in vivo*[33] and *in vitro*.[34]

Folic Acid

One aspect of folic acid metabolism might, however, be mentioned. This vitamin is in excess in the "vegan" diet, which contains unusually large amounts of green vegetables. In vitamin B_{12} deficiency, excess of folic acid can precipitate disorders of the nervous system and even lead to subacute combined degeneration of the cord.[35] It is of interest to note that nervous symptoms were encountered in a number of the vegans, who did not suffer from pernicious anemia. One of these, a boy aged 15, was found[36] to have classic subacute combined degeneration of the cord which was cured by vitamin B_{12} administered parenterally alone. In view of the occurrence of such neurologic diseases in these persons in whom the cyanide-thiocyanate metabolism seems to have been disturbed, it might be mentioned that excess of cyanide has been found[37] to produce demyelination associated with diseases of the central nervous system. However, the claim[38] of high cyanide levels in patients with demyelinating disease has not been confirmed by other workers.[39]

In conclusion, it will be realized that both of our theories involve the assumption that

hydroxocobalamin (or aquocobalamin) rather than cyanocobalamin is the active form of vitamin B_{12}.

We have put forward the above ideas not in pretence of explaining all the factors in this highly complex problem, but rather in the hope of providing a working hypothesis as a basis for discussion and further investigation.

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Reproduction and Obesity*

By E. M. Widdowson, D.Sc.

The title of this symposium is a sign of the times. If this Congress had been held 5 years ago there is little doubt that we should have been considering reproduction in relation to undernutrition, not to obesity. Both undernutrition and overnutrition can influence the capacity to reproduce successfully. The effects vary from one species to another and from one stage of reproduction to another. In some ways their influence is the same and in others the very reverse. Of the two, undernutrition is probably the more harmful and for one of the functions of reproduction it may even be an advantage to be too fat.

Professor McCance has already mentioned the organism *Tokophrya*, which lives on another unicellular organism, *Tetrahymena*. *Tokophrya* reproduces by budding off small daughter cells. If a *Tokophrya* has just the right amount of food it will produce the maximum number of embryos, that is about 12 in 24 hours. If it has too little food—say 2 or 3 *Tetrahymena* per 24 hours—it produces only 2 embryos over that time, and if it is completely starved it produces none. If, on the other hand, it is given an excess of food—say 35 *Tetrahymena* per 24 hours—it again only produces 2 embryos, and if it is allowed to feed without interruption for 24–48 hours, which it will do if given the chance, it grows to 120 times its original size and reproduction stops altogether.[38] Thus, undernutrition and overnutrition have the same effect. The deposition of too much storage material within the cell appears to prevent nuclear growth and division, and, in the absence of food, growth is impossible and nuclear division also ceases.

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In mammals reproduction is so much more complicated than in unicellular organisms that there are many different ways in which obesity or undernutrition can influence it. Female rats which have become very fat after puncture of the hypothalamus refuse to mate, even though they have a normal sex cycle.[4] This appears to be part of their general inactivity, and hereditarily obese mice behave in the same way, although they have histologically normal ovaries.[27]

Stockmen blame fatness for sterility in their animals bred for "show" purposes and there seems little doubt that there is some foundation for this belief,[35] although sound scientific evidence is singularly lacking. It has been suggested that the fat removes estrogens from the circulation by dissolving them[44] or blocks the production of progesterone.[17] There is, however, much more evidence that fertility of the female can be increased by improving the rations of livestock[36,37] than that too much food depresses it. Whether overnutrition or mild undernutrition of the male has any effect on sperm production is still an open question.[11,46]

Some clinicians blame obesity for their patients' sterility,[30] but others have been much more impressed with the fertility of fat women.[34] Some have reported that during a period of severe food shortage fewer babies are born,[1,40] whereas others have concluded that undernutrition is without effect on fertility.[28] So many psychological and physiological factors are involved, however, that even if the birth rate is reduced during a famine, it is difficult to be sure that the undernutrition has been the cause. Whatever the truth of this aspect of reproduction and nutrition, all are agreed that the obese woman is more prone to some disorders of pregnancy than the thin one, particularly hypertension, albuminuria, edema, and other evidences of toxemia.[26,34] Fetal mortality is stated by some to be

Relation of birth weight to subsequent weight.

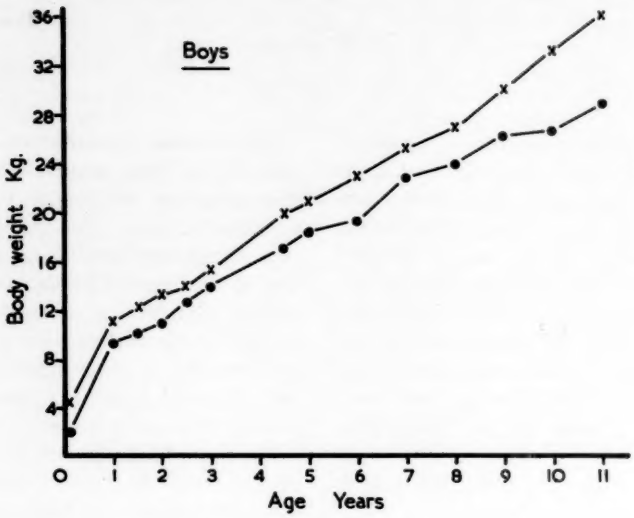

Figure 1

Relation of birth weight to subsequent weight.

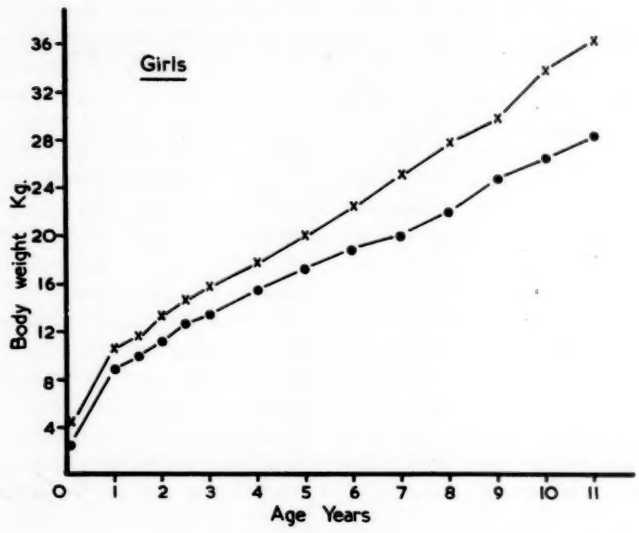

Figure 2

high,[15,26] but others have not found it to be so.[34]

There is no doubt that the fat mother tends to have a large fat baby, whether she will later develop diabetes or not.[9,15,24,26,34] Contrariwise, in a community which is suffering from a shortage of food the average birth weight of the babies is less than it is in the same community when it is well-nourished.[1,10,41]

Once a fat mother has safely brought forth a living baby, the tide turns in her favor, or certainly in favor of her offspring. She tends to produce more milk than the thin woman[39] or the undernourished one.[10] One reason for this probably is that her breasts have grown bigger, for the quantity of milk secreted appears to be directly related to the amount of mammary tissue,[25] and this is also true of the cow.[16] The big baby of the fat mother is hungry and ready to take more than the average amount of milk.[43] The child grows fast and is at all ages heavier than a child with a smaller birth weight. Illingworth and coworkers[21,22,23] have demonstrated quite clearly that the subsequent weight of children of all ages depends on the whole upon their birth weight (Figs. 1 and 2) and Mossberg[32] has shown that obese children have often been big babies at birth. A big baby born of a fat mother seems to be endowed with something which makes it continue to eat in proportion to its size. This may be growth hormone,[48] but whatever it is, it keeps these children ahead of their fellows. They eat a little more; they may or may not get fat during the growth period, but whether they do or not, they tend to reach puberty at an early age and a heavy weight.[3,5,12,32,33] This is also true of animals. An animal which grows fast and is heavier than average for its age will also attain puberty early,[7,36] and one whose diet is restricted so that its growth rate is slower than normal will attain sexual maturity late and its weight will be low at that time.[6]

As growth ceases, the big girl, with an appetite bigger than that of her companions, will continue to eat just that little bit extra each day and, as the surplus calories can no longer be expended on rapid growth, they will be laid down as fat. The bonny young woman

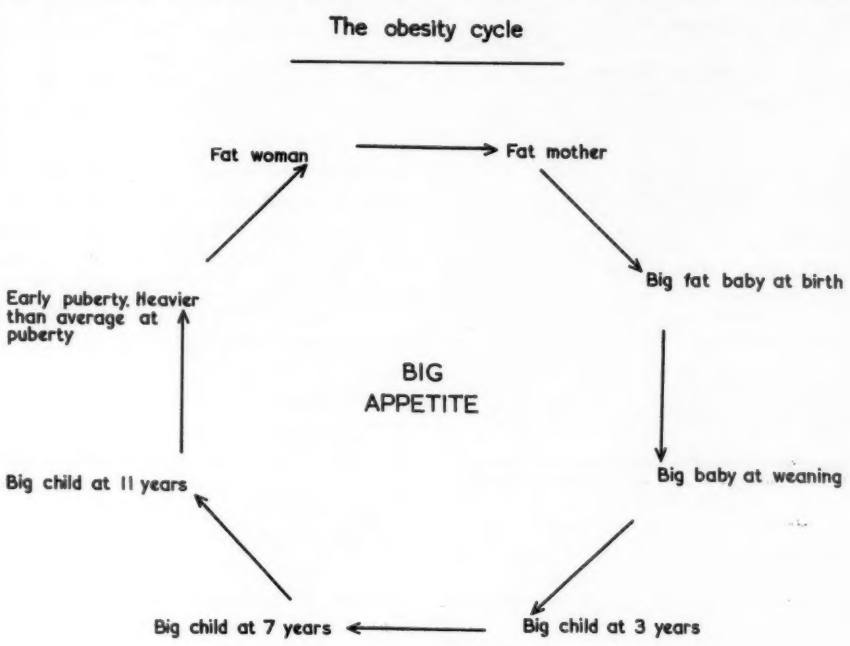
The obesity cycle
Fat woman
Fat mother
Big fat baby at birth
Early puberty. Heavier than average at puberty
BIG APPETITE
Big baby at weaning
Big child at 11 years
Big child at 7 years
Big child at 3 years

Figure 3

now only has to get married (and there seems no reason to suppose that she is less likely to do so than her thinner friend) and the obesity cycle is complete. (Fig. 3).

So far we have considered the effect of obesity on reproduction. Now I propose to look at the relationship from the other angle—the effect of reproduction on obesity. In many species it is physiological for the mother to lay down extra fat and nitrogen within her own body during pregnancy. The female rat starts to eat more food by the second day after mating, long before the fetuses can be making any measurable demands upon her.[8] She weighs more after delivery of her young than she did before she was pregnant,[2,8,46] and with each succeeding pregnancy she gains a little more weight.[8] This weight is not all lost during lactation, but no more weight is gained.[8] Morse and Schmidt[31] showed that nitrogen was retained by the rat during pregnancy and Spray[42] that both fat and protein contributed to the gain in weight (Fig. 4). Pregnant sows also store nitrogen in their own bodies during pregnancy,[13,29] but the outstanding example of the mother laying down material in her body during pregnancy is the grey Atlantic seal. The mother comes out of the sea on to the breeding territory on the shore a few days before calving, and at that time she is very fat. The newborn seal weighs about 30 pounds, and it grows to 84 pounds in two weeks—a growth rate of about 4 pounds a day. This growth is made entirely on milk, and entirely at the expense of the mother's body, for during the whole of this time the mother has nothing whatever to eat, and she loses about 200 pounds of her own weight during these two weeks.[14] Incidentally, the bull seals also put on a great deal of fat before breeding time, and they too go without food for several weeks while they are lying on the shore waiting for the females to bring forth their young and rear them and be ready for mating again about 14 days after the birth of the calf.

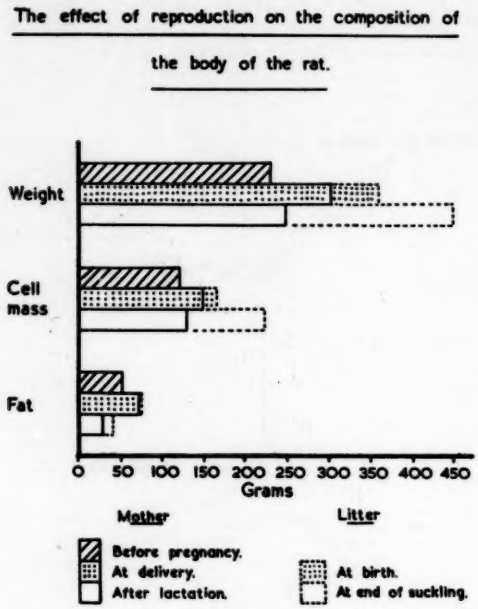

Figure 4

The human mother also normally stores more nitrogen and possibly also more fat in her own body during pregnancy than is required for the development of her baby.[18,19,20] In order to do this she must eat more food, and sometimes she carries this to excess and becomes very fat. Sheldon[39] has investigated the problem of maternal obesity and has found that some women put on weight with each succeeding pregnancy, while others put on a great deal of weight after one particular pregnancy. Sometimes the obesity does not develop until immediately after delivery or during lactation. In any case, this maternal obesity is always due to over-eating, and I would like to suggest that the woman who becomes fat during pregnancy or lactation has been travelling round the obesity cycle all her life. She was herself a big baby at birth and she will produce a big baby and lactate well.[39] She is always potentially fat, but the obesity first becomes gross when she herself reproduces.

One point remains to be considered: the effect of obesity on the length of reproductive life. We have been told today that obesity shortens the expectation of life. How does it affect reproductive life? The overweight woman or animal begins her reproductive life early. Does she wear out early? In mice an

unlimited supply of food seems to shorten reproductive life,[45] but in women, as far as I know, we have no evidence one way or the other. I can only conclude by quoting the words of the psalmist, "They shall bring forth fruit in old age; they shall be fat and flourishing." (Ps. 92. 14.)

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Philosophy in Medicine

"Galen desired that every true physician should be also a philosopher. Philosophical enquiry in medicine is apt to be regarded as an arduous eccentricity for which few physicians in our time have had either the opportunity or the inclination. Yet it is a worth-while pursuit, for a knowledge of the history of ideas has a moderating influence. It helps to keep a balance between undue dogmatism on the one hand and undue scepticism on the other; and above all in revealing the thoughts and expounding the works of some of the greatest minds in human history, it inculcates a humility which is the surest shield against intellectual arrogance."
—Sir Henry Cohen. *Proceedings of the Royal Society of Medicine* 48: 155, 1955.

Testing for "Guts"

"There were enthusiasts who believed that the nutritional state of a child could be measured by the length of time for which he could hang by his hands from an overhead bar or by the strength he could record on the dial of a dynamometer as he squeezed a spring grip or pulled a handle. The fallacies were fairly soon obvious. The 'hanging bar' test measured not only muscular power but tenacity of purpose and that very ill-defined quality, 'guts.' The dynamometer measured not only strength but skill and knack in the use of muscles. For that reason, the nutritionists abandoned these tests. . . . In our view that abandoning of functional tests was premature, for these adventitious qualities, 'guts,' 'muscular knack,' and 'fitness,' though hard to define and often hard to explain, were certainly parts of total health in that the person who had them in a high degree was certainly a better human being than the person who lacked them."
—*The Medical Press* 232: 533, 1954

Blood Lipid Levels as Influenced by Weight Reduction in Men

By N. S. Moore, M.D.,* J. H. Fryer, M.S., B.S.,† C. M. Young, PH.D.,‡ and L. A. Maynard, PH.D.§

In an earlier paper observations were reported on blood lipid levels in twenty-four women while losing 15 to 60 pounds of weight at the rate of from 0.6 to 2 pounds per week; similar blood lipid data for twelve women who did not lose weight were also presented. The data as a whole revealed the rather wide fluctuations in blood lipids which occur in women whether losing weight under dietary control or changing little in weight with no dietary control. No detectable linear relation between weight change and level of total serum lipids within individuals was found. A small but significant overall linear relation within individuals was noted between weight loss and serum phospholipids, and between weight loss and serum cholesterol.[1]

Methods

The study presented here deals with repeated observations on blood lipid values for twenty-four men from 10 to 96 per cent overweight, all of whom lost weight. The "per cent overweight" was calculated by the formula of Showacre, which uses height-chest diameter ratios to complement standard height-body build-weight tables.[2] Twelve men were under rigid dietary control for a period of nine weeks. These men ate all their meals at a diet table operated by the research team. One investigator supervised menus and a weekly weigh-in period. Fasting blood samples for lipids were taken at the beginning and end of each three-week period and weekly while the subjects were on a diet of 50 g of fat per day; also, samples were taken weekly during the three-week period after the fat in the diet had been restored to 103 g per day. The remaining 12 men, not under rigid dietary control, were observed from 11 to 24 weeks (average 15.5 weeks).

Group I consisted of twelve men who were observed in a nutrition clinic sponsored jointly by the local medical society, the county health department, and the School of Nutrition. The prescribed caloric intake of this group was 1400 calories, with a daily intake of 80 g fat. The ages in this group varied from 31 to 68 years, with an average age of 42 years. Members of Group I lost from 7 to 42 pounds each over a period varying from 11 to 23 weeks at a rate of loss of from 0.5 to 1.8 pounds per week.

Group II was composed of twelve men who were on a carefully controlled weight reduction program over a period of nine weeks, during which time the fat content of the diet was 103 g per day for the first and last three-week periods, and 50 g per day during the middle three-week interval. The ages in this group varied from 19 to 28 years, the average being 22 years. This group lost from 13.5 to 31.5 pounds each over the nine-week period, at a rate of loss varying from 1.5 to 3.5 pounds per week.

The data for total lipids presented here represent the combined total fatty acids and cholesterol as determined by the method of Bloor.[3] The phospholipids were obtained by determining phosphorus in the lipid extract

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TABLE I

Serum Lipid Values in Twenty-four Men who Lost Weight

% Overweight	Rt. of wt. loss	Total Lipids				Phospholipids				Cholesterol			
		Initial	Term.	High	Low	Initial	Term.	High	Low	Initial	Term.	High	Low
%	*lb/wk*	*mg/100 ml*				*mg/100 ml*				*mg/100 ml*			
Group I													
63	1.83	712	474	712	474	270	207	270	207	180	148	185	148
47	1.54	684	615	684	615	260	242	260	222	200	203	234	200
20	1.50	615	455	615	455	255	212	255	208	235	172	235	172
20	1.40	740	460	740	460	258	192	258	192	216	168	216	168
45	1.36	687	588	687	588	304	215	304	215	211	182	211	172
47	1.27	730	625	730	625	230	222	247	222	204	205	227	203
29	1.14	522	680	680	522	242	254	275	242	196	211	211	196
20	0.92	544	592	665	544	222	255	255	222	220	240	240	210
96	0.88	995	719	995	719	336	264	336	264	259	220	259	220
45	0.85	625	635	635	480	210	228	228	195	190	184	190	174
45	0.78	825	685	825	662	300	258	300	215	246	237	246	221
19	0.47	692	782	782	563	302	324	324	277	251	234	251	209
Group II													
48	3.50	642	830	888	608	286	283	286	196	232	240	251	185
12	3.22	491	680	820	491	200	252	262	198	154	204	250	154
27	2.91	751	590	751	562	286	230	286	199	269	187	269	149
31	2.86	622	695	755	595	234	214	290	206	209	190	212	184
15	2.83	566	525	730	450	224	221	224	166	178	152	196	142
25	2.80	610	690	835	610	257	226	262	216	209	200	209	167
23	2.63	661	750	860	625	260	243	264	215	225	200	261	195
22	2.52	520	530	670	488	223	168	223	155	164	132	180	127
22	2.22	490	403	670	403	196	164	207	163	155	117	155	117
18	2.19	677	659	860	612	238	206	238	183	215	174	215	157
20	2.16	528	522	765	500	230	198	230	186	181	167	193	144
10	1.50	618	795	865	618	304	307	307	228	223	276	276	215

by the method of Sumner.[4] The total cholesterol was obtained by the method of Sperry and Webb.[5]

Results

In Table I, the initial and terminal values of serum total lipids, serum phospholipids, and serum cholesterol are given for both groups of men while losing weight. The data also show the high and low values during the observation period for each of the serum lipids.

Of the twelve subjects in Group I, four had terminal serum total lipid values higher and eight lower than initial values. Of the nine subjects having initial-terminal differences greater than 10 per cent, two had higher (and seven had lower) terminal serum total lipid values. Serum total lipid values between the initial and terminal levels in this group showed wide fluctuation from their means. A regression analysis* of serum total lipids on weight in this group shows a "within subjects" regression coefficient of 5.50, or a decrease of 5.5 mg serum total lipids per 100 ml serum per pound of body weight lost. This is significant at the 1 per cent level (Table II).

The men in Group II showed serum total lipid values different from the men in Group I. In Group II, seven subjects had higher (and five subjects had lower) terminal serum total lipid values. Eight subjects had initial-terminal differences greater than 10 per cent; of these, six had higher and two had lower terminal serum total lipid values. No relationship was found between the rate of weight loss and the degree of change of initial-terminal values. The "within individuals" regression coefficient of serum total lipids on weight of Group II was 3.98, or an increase of 3.98 mg serum total lipids per 100 ml serum per pound of weight lost. This is significant at the 1 per cent level.

Phospholipids

Of the twelve subjects in Group I, four had higher and eight had lower terminals than initial serum phospholipid values. Of the seven subjects having differences greater than 10 per cent, only one had a higher terminal phospholipid serum level, while six had lower terminal serum values. Rate of weight loss was not a factor in the initial and terminal serum phospholipid values. (Table I).

Regression analysis shows that the "within individuals" regression coefficient was 1.77, or a decrease of 1.77 mg phospholipids per 100 ml serum per pound of weight lost, and was significant at the 1 per cent level (Table II).

Of the 12 subjects in Group II, two had higher but ten had lower terminal serum phospholipid levels. Of those having initial-terminal differences greater than 10 per cent, one showed a higher and six showed lower terminal values. Group II followed the same pattern as Group I in regard to the number and direction of those with differences greater than 10 per cent in initial and terminal values. The "within individuals" regression coefficient for this group was 0.58, or a decrease of 0.58 mg phospholipids per 100 ml serum per pound of body weight lost. However, the regression coefficient was not significant.

Cholesterol

In Group I, there were four subjects who showed higher and eight who showed lower terminal serum cholesterol levels. However, there were but five men who had initial-terminal differences of 10 per cent or more, and all of these had lower terminal serum cholesterol values. Regression analysis shows a "within individuals" regression coefficient of 0.89 (significant at the 1 per cent level) or a decrease of 0.89 mg cholesterol per 100 ml serum per pound of weight lost.

TABLE II

Regression Coefficients of Components on Weights of 24 Women Losing Weight[1] and 24 Men Losing Weight

Component	Women (N = 24)	Men (N = 24) Group I (N = 12)	Men (N = 24) Group II (N = 12)
Total lipids	0.33	5.5†	−3.98†
Phospholipids	−0.64*	1.77†	0.58
Cholesterol	−0.58*	0.89†	0.24

* Significant at 5% level.
† Significant at 1% level.

*The authors are indebted to Dr. R. G. D. Steel of the Biometrics Unit, Department of Plant Breeding, for statistical analysis of total lipids, phospholipid, and cholesterol data.

In Group II, three subjects showed higher, while nine showed lower, serum cholesterol terminal levels over initial values. In the eight men showing differences of more than 10 per cent, two had higher and six had lower terminal serum cholesterol values.

If both Groups I and II are considered together, there were two with higher but eleven with lower terminal serum cholesterol values among the thirteen subjects who had initial-terminal serum cholesterol level differences above 10 per cent. A regression analysis of Group II shows a "within subjects" regression coefficient of 0.24 (not significant), or a decrease of 0.24 mg cholesterol per 100 ml serum per pound of body weight lost.

Discussion

Physiologically, the two groups of twelve males each in this study differed in average age, duration of the reduction period, in rate of loss of weight and the amount of fat in the diet. Both groups were "healthy," no one in either group showing symptoms or signs of disease. The groups differed statistically in the following ways: Linear regression coefficients showed statistical differences in the two groups. Whereas the regression analysis in Group I, the older men, showed a 5.5 mg decrease in serum total lipids per 100 ml serum per pound of body weight lost, that for Group II, the younger men, was a 3.98 mg increase in total lipids per 100 ml serum per pound of weight lost.

For phospholipids, the regression coefficient for Group I was significant at the 1 per cent level. This was not true for Group II. No valid comparison of regression coefficients for cholesterol can be made because of the nonhomogeneity of regression coefficients in either group.

It is apparent, therefore, from the physiologic and statistical differences of the two groups, that data from each group cannot be pooled and the totals compared with data obtained by us from women who lost weight. The two groups of men had different patterns of total lipid, serum phospholipid, and cholesterol behavior during weight loss than were observed in women. The data for Table II indicate there is no detectable linear relation between weight change and level of serum total lipids within individuals for women, whereas a linear relationship is indicated for men. Although linear relationships between weight change and blood phospholipid and cholesterol levels are shown in Table II for both men and women, the regression coefficients are in opposite directions for each sex, respectively, during weight loss.

While the study includes comparatively few subjects, and while there seem to be certain linear relationships shown, the question remains:—*Is the relationship between the level of blood lipids and weight loss of physiologic importance?* In the case of phospholipids and cholesterol for both men and women, the answer would appear to be no—for wide fluctuations in these values have been observed in normal people both losing and not losing weight. In the average overweight person, loss of all excess weight through dieting would not change blood lipid values to the extent they would exceed the variations in these lipids occurring in the serum of people of normal weight.

In Group II, the wide fluctuations in blood lipids appear to be partly related to the content of fat in the diet, for in every subject the lowest levels of serum lipids were obtained at the end of a three-week period when the fat content of the diet had been lowered from 103 to 50 g daily. In Figure 1, the mean blood lipid values and weight curve of the twelve men in Group II are shown. Two weeks following reduction of fat in the diet, the level of all blood lipids which were measured started to fall and were lowest at the end of the low fat period one week later. After one week on an uncontrolled diet, the curves again rose. The slope of the weight curve during this time remained constant. This observation is in line with that previously reported in the literature.

The rather high regression coefficient for total lipids in opposite directions for the older and younger male groups, respectively, would appear to be of questionable conse-

quence. That wide fluctuations occur in serum total lipids in people of constant weight is well known; therefore, a similar reaction in men losing weight appears not to be of physiologic importance.[1,6]

wide fluctuation in blood lipids which occurs in men losing weight.

The data from the two groups could not be pooled and compared with similar data from women losing weight because of the physio-

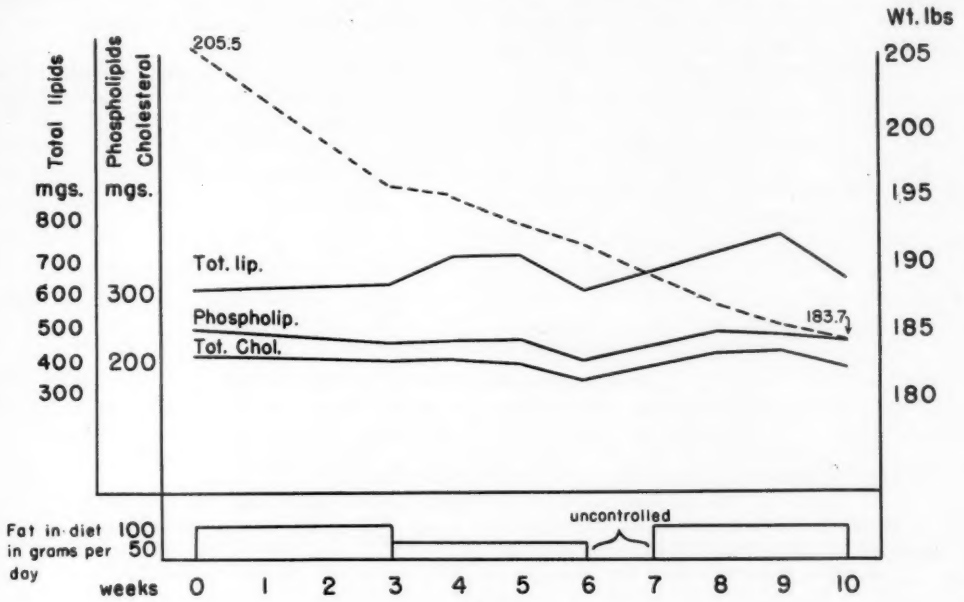

Fig. 1
Group II

Mean blood lipid values of 12 men on controlled diet. Amount of fat in diet during each period and mean weight loss values.

Summary

Blood lipid data are presented for twenty-four men while losing from 0.5 to 3.5 lb body weight per week. Twelve of these individuals, Group I, ranged from 31 to 68 years of age (average age 42 years); twelve other men, Group II, ranged from 19 to 28 years of age (average age 22 years).

The men in Group I were not under rigid dietary control, but had diets prescribed for them which contained 80 g of fat per day. The subjects in Group II were under rigid dietary control. Their daily intake of fat was 103 g for the initial and terminal three weeks' periods and 50 g of fat daily during the middle three weeks' period.

The data as a whole revealed the rather logic and statistical differences of the groups. However, both Groups I and II showed different regression coefficients for blood lipids than did women losing weight, i.e., women showed a small increase in serum phospholipid and cholesterol per pound of weight lost, but both groups of men showed decreases with weight loss. While in the women there was no detectable linear relationship between weight change and serum total lipids (within individuals), in both groups of the men there were highly significant linear relationships. In the older men there was a decrease and in the younger men an increase in serum total lipids with weight loss.

Even though regression analyses establish regression coefficients for men losing weight

which are different from those for women losing weight, the resulting changes in the level of serum lipids appear to be of no physiologic importance.

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Old Men and Their Food

"It is rarely . . . that one comes across a contribution at once so informative, interesting and even amusing, as the recent report by the Senior Almoner of the Belfast City Hospital on elderly men in common lodgings, published by the Nuffield Provincial Hospitals Trust. This deals with nearly four hundred men in some sixteen lodging houses scattered all over Belfast. . . .

"Many of the old men, as might be expected, had arrived at very decided if somewhat bizarre views on the subject of diet. 'The elderly man who remarked spryly: "We ould fellas don't have the same use for food now," summed up this situation very well. One particularly rubicond old gentleman attributed his good health to cod-liver oil which he spread on bread instead of butter and washed down with spring water specially carried down by himself in bottles from the upper reaches of the River Lagan. Another was a firm believer in cabbages and herrings which he always ate together and which he bought in large quantities and stored happily in his locker from one pension day to the next. A wizened-faced old man was scornful of sausages and referred to them as "those mysteries," a view which was held by most of his fellow lodgers; while a seedy, taciturn old man with no socks or dentures, announced that tinned soup, bread, tea and frys were his main dishes, though he confessed that his mother had taught him how to make "pies and puddens." These four items plus an occasional helping of potatoes, porridge and broth, constituted the main items of diet for those elderly men who took the trouble to cook. . . . The broth gang has already been mentioned and is the answer either for the inexperienced cook or the old man who cannot be bothered to make a proper meal for himself. Each member of the gang contributes an offering to the pot and it was surprising the number of old men who have contacts in the market or who are "in the way of getting" the old carrot, turnip or onion. In this manner a large pot of broth can be made by the acknowledged expert and is ladled out to serve as dinners for several days.' "

—*The Medical Press* 232: 326, 1954.

Some Relations Between Body Weight, Body Fat, and Calorie Intake

By MORTON I. GROSSMAN, M.D., PH.D., AND HARRY S. SLOANE

THE RELATION between the weight, composition, and configuration of the body and basal metabolic rate has been studied extensively. Likewise, considerable attention has been given to the relation between body size and the energy cost of specific tasks. However, aside from certain theoretical considerations, little information is available on the relation between total energy requirement and body size and body composition.

In the course of studies conducted by the Medical Nutrition Laboratory at Pole Mountain, Wyo., during January-March 1953, information bearing on this problem became available and certain aspects of it are presented in this report.

OBSERVATIONS AND RESULTS

The detailed description of the procedures used is given in the report of the experiment.[1] In essence, observations are available on total food intake, body weight, body fat as determined by skinfold measurements, and urine total nitrogen output on a group of 87 test subjects living under controlled conditions. The weight of each food item eaten by each man at each meal was recorded. The caloric content of the food eaten was calculated by the use of standard tabular values.

THE RELATION OF CALORIE INTAKE TO BODY WEIGHT

During a one-week period (the sixth week of the experiment) when the men were on an *ad libitum* food intake (with a maximum of 4100 calories available), there were 35 men who showed less than 0.5 per cent change in body weight. The activity program during this week was not regulated. In the 35 men with constant body weight, the relation between body weight and calorie intake was analyzed statistically. The correlation coefficient between these variables was +0.303, which is just below that required for significance at the 5 per cent level.

These same data were also analyzed after transformation of body weight and calorie intake values to logarithms. The correlation coefficient was +0.314, which again is just below the value required for significance at the 5 per cent level.

A similar analysis was performed on the data from the third week of the experiment, at which time the men were on an assigned program of activity designed to produce an average expenditure of 3700 calories per day. Food intake was *ad libitum* with a maximum of 4100 calories per day available. There were 52 men whose body weight changed less than 1 per cent during this week. The correlation coefficient was +0.299 (significant at the 5 per cent level).

The data from the third week were also calculated in the logarithmic form. The correlation coefficient was +0.327 (significant at the 5 per cent level of probability).

Comment

The attempt to correlate body weight with calorie requirements is complicated by variations in calorie expenditure for physical activity. Obviously, physical activity will vary even during a period when a prescribed program is being followed. However, in the practical situation, the aim is to adjust for body weight within a group of subjects who are all at the same general level of activity.

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The opinions expressed in this paper are those of the authors and do not necessarily represent those of any governmental agency.

We have been unable to find other data in the literature relating calorie intake to body weight, and our own observations are admittedly limited. The correlation between body weight and calorie intake, although significant, was low. Further observations are obviously required to clarify the relation of body size to total energy requirement.

The Relation of Calorie Intake to Body Weight and Fatness

The same data from the sixth week referred to above were used, but, in addition, the per cent body fat as calculated from skinfold measurements was also incorporated and multiple regression analysis performed. For the regression of calorie intake on body weight and per cent body fat, the multiple regression coefficient, R, was 0.496, which is significant at the 5 per cent level of probability. The partial correlation coefficient of calorie intake and body weight independent of fatness, was +0.466, which is significant at the 1 per cent level. The partial correlation coefficient of calorie intake and per cent body fat, independent of body weight, was —0.412, which is significant at the 5 per cent level.

The data from the third week referred to above were also subjected to multiple regression analysis. The multiple correlation coefficient, R, was 0.494 (significant at the 5 per cent level). The partial correlation coefficients were +0.494 (significant at the 1 per cent level) for calorie intake and body weight independent of fatness, and —0.412 (significant at the 1 per cent level) for calorie intake and per cent body fat independent of body weight.

Comment

It is only within recent years that methods for the accurate estimation of leanness-fatness in man have become available.[2] To our knowledge, no previous studies have been performed utilizing these methods to correlate body fatness and calorie intake. The only studies available on the relation of fatness to food intake are comparisons of normal individuals with the grossly obese (see reference No. 3 for citations to literature). The most recent study of this type was that of Beaudoin and Mayer,[3] who found that obese women consumed more calories than nonobese controls, but since they give no height-weight data on their subjects, it is difficult to evaluate their findings.

For the estimation of body fatness from skinfold thickness in the present study the regression equations derived by Brožek and Keys were used.[4] More recent studies in our laboratory[5] have shown that, while the correlation between fatness, as estimated by skinfold thickness using the Brožek-Keys equation, and by underwater weighing using the Pace-Rathburn equation, is excellent ($r = +0.92$), the skinfold method consistently underestimates the body fatness by approximately 5 per cent. Therefore, the values for per cent body fat reported here are probably lower than the true values, but the relative values for comparisons among different men are probably valid.

The results obtained indicate that for a given body weight the calorie intake decreases with increasing body fatness. The most obvious explanation for this relation is that as the body fatness increases, the fraction of metabolically active tissue decreases. Whether this is the entire explanation for this phenomenon remains to be established. The possibility that a correlation exists between fatness and the amount of activity in which the individual engages should also be considered.

Peckos[6] reported that children with endomorphic physique consumed less calories than mesomorphs or ectomorphs, a finding in keeping with the present results.

Relation of Per Cent Body Fat to Per Cent of Calories in the Diet Derived from Fat

Again, using data from the sixth week, but in this instance including 87 subjects, i.e., both those whose weight remained constant and those whose weight changed, a correlation coefficient was computed between per cent body fat and per cent of calories derived from fat. The correlation coefficient was +0.217,

which is significant at the 5 per cent level of probability.

A similar analysis for the third week of the experiment on 85 subjects gave a correlation coefficient between per cent body fat and per cent of calories derived from fat of +0.117, which is not significant at the 5 per cent level.

Comment

Studies in animals have rather clearly established that the per cent of fat in the body increases as the per cent of fat in the diet increases,[7-9] although there would appear to be differences among strains of animals in this regard.[9] Studies of the diets selected by obese human subjects have not revealed a clear difference in the per cent of calories derived from fat when compared to nonobese controls.[3] In the present study, the correlation between per cent of calories derived from fat and body fatness was significant in one period, and just below the 5 per cent level of significance in another period. Since the diet offered was a fixed one, relatively little opportunity was afforded for variation of per cent of calories derived from fat by selection. The suggestive findings in the present study indicate that further studies under more nearly *ad libitum* conditions of selection might give more clear-cut correlations.

Relation of Weight Loss During Calorie Restriction to Body Weight and Per Cent Body Fat

During the seventh to tenth weeks, inclusive, the subjects were placed on restricted calorie intakes (2500 calories during the first 11 days and 2000 calories during the last 12 days). Sixty-two of the subjects consumed all the food offered to them during this period. Multiple regression analysis of weight loss on body weight and per cent body fat at the beginning of the restricted calorie period gave a multiple correlation coefficient, R, of 0.605, which is significant at the 1 per cent level of probability. The partial correlation coefficients were +0.472 (significant at 1 per cent level) for weight loss and initial body weight independent of fatness, and +0.14 (not significant at 5 per cent level) for weight loss and per cent body fat independent of body weight. The mean weight loss was 3.97 ±1.24 (S.D.) kg.

Comment

It was found that weight loss during calorie restriction at a fixed level was strongly correlated with initial body weight, as would be anticipated. Surprisingly, the weight loss was not significantly correlated with body fatness. *A priori,* one would anticipate that weight loss would be less with higher per cent fatness, for two reasons, viz., (1) as demonstrated in the present study, for a given body weight the calorie requirement decreases as fatness increases, and (2) the high calorie value of adipose tissue would require greater calorie deficit per kilogram of loss. However, in regard to this second point, Keys and Brožek[6] have pointed out that the tissue lost during calorie restrictions tends to have a relatively constant composition when subjects in the normal range of body fatness undergo slight weight reduction.

Relation Between Nitrogen Excretion During Calorie Restriction and Body Weight and Per Cent Body Fat

Again using data from the period of restricted calorie intake (seventh through tenth weeks of the experiment), the relation between urine nitrogen output in grams per day on the fifth day of calorie restriction, body weight in kg, and per cent body fat, both of the latter two values being taken at the beginning of caloric restriction, was analyzed by multiple regression techniques. The multiple correlation coefficient was 0.429 (significant at the 1 per cent level). The partial correlation coefficients were +0.425 (significant at the 1 per cent level) for urine nitrogen and body weight independent of fatness, and −0.361 (significant at the 1 per cent level) for urine nitrogen and per cent body fat independent of body weight. The mean urine nitrogen was 12.35 ±2.76 (S.D.) grams per day.

A similar analysis was performed on the data obtained later in the period of calorie

TABLE I

Means and Standard Deviations

Analysis No.	Week		Description	Mean & standard deviation	n
I	6th	Y	Calorie Intake	3645.6 ± 328.5	35
		X	Body Weight (kg)	70.1 ± 7.61	
II	6th	Y	Calorie Intake	3645.6 ± 328.5	35
		X	Body Weight (kg)	70.1 ± 7.61	
III	3rd	Y	Calorie Intake	3583.6 ± 408.6	52
		X	Body Weight (kg)	71.0 ± 10.72	
IV	3rd	Y	Calorie Intake	3583.6 ± 408.6	52
		X	Body Weight (kg)	71.0 ± 10.72	
V	6th	Y	Calorie Intake	3645.6 ± 328.5	35
		X_1	Body Weight (kg)	70.1 ± 7.61	
		X_2	% Body Fat	4.88 ± 2.69	
VI	3rd	Y	Calorie Intake	3583.6 ± 408.6	52
		X_1	Body Weight (kg)	71.0 ± 10.72	
		X_2	% Body Fat	5.95 ± 3.73	
VII	7th–10th	Y	Weight Loss	3.97 ± 1.24	62
		X_1	Body Weight (kg)	69.93 ± 8.34	
		X_2	% Body Fat	4.55 ± 2.72	
VIII	7th	Y	Urine nitrogen output in grams per day	12.35 ± 2.76	62
		X_1	Body Weight (kg)	35.55 ± 4.51	
		X_2	% Body Fat	2.53 ± 1.51	
IX	9th	Y	Urine Nitrogen	11.71 ± 2.21	75
		X_1	Body Weight (kg)	68.67 ± 8.65	
		X_2	% Body Fat	3.36 ± 1.90	

restriction, using urine nitrogen in grams per day on the fifteenth day of calorie restriction, body weight in kg on the fifteenth day of calorie restriction, and per cent body fat on the twentieth day of calorie restriction. The multiple correlation coefficient was 0.242 (not significant at 5 per cent level). The partial correlation coefficients were +0.239 (significant at 5 per cent level) for urine nitrogen and body weight independent of fatness, and −0.194 (not significant at 5 per cent level) for urine nitrogen and per cent body fat independent of body weight.

Comment

Studies on obese subjects[10,11] have revealed that during calorie restriction they exhibit a smaller negativity of nitrogen balance than do nonobese subjects. In the present study, this relation was found to obtain also among men within the normal range of leanness-fatness, that is, nitrogen excretion decreased as body fatness increased. This would appear to cast some doubt upon the concept expressed above, that the composition of tissue lost during calorie restriction does not vary with initial fatness. However, the correlation between body fatness and nitrogen excretion which obtained early in the course of calorie restriction was not present later.

A summary of the means and standard deviations for the various sets of data is given in Table I. The details of the statistical analyses are contained in a report from this laboratory.[12]

Discussion

The calorie requirements of an individual can be divided into the following components: basal metabolism, specific dynamic action, and energy expenditure involved in physical activity. An expression of the form,

$$\text{basal metabolism} = aW^n$$

predicts basal metabolism with reasonable accuracy (where a and n are constants and W is body weight). The n constant is usually taken to be 0.73 (or a value of this general magni-

tude) and the a constant varies with sex and age. Specific dynamic action is in general related to the amount of food eaten and is usually taken as 0.66 to 0.10 times the calorie value of food eaten. The energy expenditure involved in physical activity is usually divided into two components, one related to and directly proportional to body size, and another relatively unrelated to body size. The FAO Committee on Calorie Requirements,[13] from a theoretical analysis of these components, arrived at the conclusion that total energy requirement for people of various sizes and activities could be estimated with reasonable accuracy from an equation of the same form as that relating body weight and basal metabolism, namely $aW^{0.73}$, by selecting a value of a appropriate for the activity level of the group. This is essentially the same as the frequently employed method of estimating energy requirement by multiplying basal metabolism by a factor appropriate for the activity of the individual.[14] As a test of the validity of this equation we have calculated the a and n constants from our own data. For men with an average body weight of 71 kg and an average calorie intake of 3600, the equation in the form suggested by the FAO Committee is $\hat{y} = 163W^{0.73}$, whereas the corresponding equation derived directly from our data for the third week of the experiment is $\hat{y} = 1168.5W^{0.262}$. When the variances of the observed calorie intakes from the regression lines described by these two equations are compared, a variance ratio, or F value, of 1.53 is obtained (not significant at the 5 per cent level of probability). Therefore it cannot be stated with certainty that the equation derived directly from the data has better predictive value than the theoretically derived equation of the FAO Committee. Perhaps the point of greatest interest is that the correlation between calorie intake and body weight, although statistically significant, is of low order, and that accurate prediction of calorie requirement requires the consideration of parameters in addition to body weight. As pointed out elsewhere in this paper, body fatness is one such factor, and taking it into account improves the correlation between body weight and calorie intake.

Summary

During the course of a field experiment in which 87 soldiers served as test subjects, the following observations were made:

1. The correlation between body weight and calorie intake, although statistically significant, was low.

2. When calorie intake was related to per cent body fat independent of body weight, an inverse relationship was revealed, i.e., calorie intake decreased as per cent body fat increased.

3. During two one-week periods the relation between per cent body fat and per cent of calories derived from fat was examined. In one instance a significant positive correlation was found. In the other instance the correlation was not significant.

4. The loss of body weight during a period of calorie restriction at a fixed level was found to be significantly correlated with initial body weight but not with initial body fatness.

5. Nitrogen excretion in the urine early during the period of calorie restriction was significantly positively correlated with body weight and significantly negatively correlated with per cent body fat. At a later time during calorie restriction nitrogen excretion was correlated with body weight but not with body fatness.

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The Patient's Expectations

"The average patient appears to expect one of four things from his doctor: (1) some form of medicine, (2) advice or guidance in connection with a particular action or event going on at the time he is attending, (3) support (usually against some member of the family) in some particular action, or (4) "a letter to see a specialist." In addition all want to be told "what is the matter" and, of course, to be reassured that the condition is not serious.

Yet at the end of the first consultation most are content to go away without any of these things if they have an appointment to come again. Despite the usual cry, "The patient expects a bottle of medicine," I have found that if he is given ample time and the opportunity to talk fully, and is thoroughly examined before being told it is necessary to seek the cause of his symptoms by means of further interviews, he is happy to accept this—and there is seldom any later demand for medicine. Similarly, though it is said that the public has become "hospital-conscious" or "specialist-minded," I find that most patients who ask for "a letter to see a specialist" will agree to attend for further discussion of their troubles once they have been encouraged to talk freely."

—P. Hopkins. *Medical World* 83: 20, 1955.

Serum Vitamin A Level: A Critique of Methods and Significance

By W. O. Caster, ph.d.,* and Olaf Mickelsen, ph.d.†

It is generally believed that serum vitamin A levels in human beings reflect the nutritional status with respect to that vitamin. For this reason, serum vitamin A levels have been determined in many nutritional studies. These studies were both long-term experiments on small groups of subjects and surveys of large population samples.

A micromethod[1] has been developed which permits the determination of vitamin A in a drop of blood. The technique has been used in an increasing number of studies. For this reason, it is appropriate to examine the method for its accuracy and consistency. At the same time, the utility of the serum vitamin A level as an index of nutritional status is evaluated on the basis of a number of studies that have already appeared.

Chemical Considerations

The spectrophotometric method for the determination of vitamin A is rapid and easy, but it is beset by the fact that other biological substances may interfere with it to a greater or lesser extent. A hypothetical example of this is depicted graphically in Figure 1. The upper line, ABC, shows the typical absorption peak of vitamin A around 328 mμ. Line DEF represents the absorption curve of non-vitamin A material absorbing in this same spectral region. Thus, B, the absorbance at 328 mμ, is due not only to the presence of vitamin A (cross-hatched area), but is the sum of this and E, the absorbance due to non-vitamin A compounds. The problem then is to determine the value of B minus E, or the absorbance at 328 mμ which is due to vitamin A. The first part of this paper will consider two different approaches to the problem: (1) The chemical method of Bessey *et al.*[1] by which both points B and E are measured directly, and (2) the mathematical procedure of Morton and Stubbs[2] in which points A, B, and C are determined and point E is calculated.

In the vitamin A micromethod of Bessey *et al.*,[1] point B is first determined directly on a kerosene-xylene extract of hydrolyzed serum. To obtain point E, this extract is irradiated with ultraviolet light to destroy the vitamin A and another spectrophotometric reading is made at 328 mμ. The difference in spectral

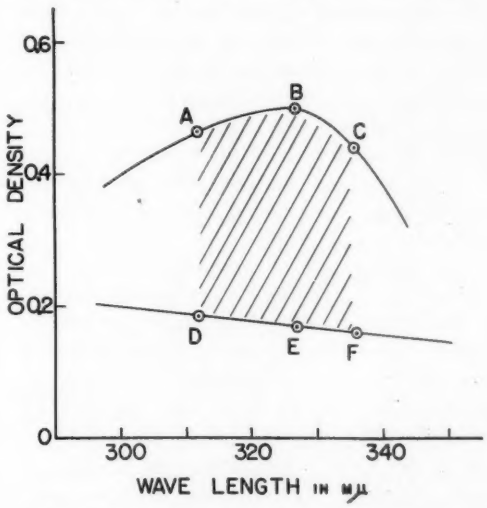

Fig. 1. A representation of the conditions which are assumed to exist in the typical spectrophotometric determination of vitamin A in serum. See text for discussion.

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absorbance before and after irradiation (that is, B minus E) is multiplied by an empirical factor and the result reported as vitamin A concentration. These authors[1] presented data to show that the method is capable of giving serum vitamin A values with a precision of about $\sigma = 2$ per cent, that these vitamin A values agree within $\sigma = 8$ per cent with values obtained by the Carr-Price chemical method, and that the absorbance differences between 300 mμ and 400 mμ resulting from the destructive irradiation of the extract closely approximate the values obtained by the irradiation of pure vitamin A solutions. The work of Little[3] is cited as further proof of the validity of this method.

The macro version of this vitamin A procedure has been reviewed in some detail.[4–6] Demarest[5] has pointed out that the validity of the destructive irradiation procedure depends upon three assumptions: (a) Irradiation with ultraviolet light converts vitamin A to products which have no appreciable absorbance in the vicinity of 328 mμ. (b) Other ultraviolet absorbing substances present in the serum extract do not undergo appreciable change in their absorbance at 328 mμ during irradiation. (c) Irradiation causes a quantitative destruction of vitamin A.

It is reported[1,3,6] that the first and third conditions are satisfied in the analysis of a variety of fish oils and in the analysis of serum and tissue extracts. Compliance with the second condition is a little more difficult to check. In work with serum, where the amount of non-vitamin A material absorbing at 328 mμ is large, the second condition becomes particularly important.

The second method to be considered is that of Morton and Stubbs.[2] Their mathematical procedure for treating the spectrophotometric data has been accepted as the basis for official methods.[7,8] In this procedure, points A, B, and C in Figure 1 are determined and E is calculated. This method depends upon the fact that if one knows the shape of the absorption curve for pure vitamin A and is given point A or C, he can calculate B. Or, what is more important, if he is given A, B, and C, he can calculate where line DEF must be in order that the ratios (A—D)/(B—E) and (C—F)/(B—E) shall be characteristic of pure vitamin A. Implicit in this calculation is the assumption that line DEF (the absorption curve of the non-vitamin A material) is linear in this spectral region. For the details of the mathematics the reader is referred to the original work.[2] The nomograph of Oser[9] speeds these calculations and has been used in the present work. Suffice it to say that, given points A, B, and C, this method allows one to compute the proportion of the absorption at 328 mμ which is due to vitamin A and the proportion which is due to non-vitamin A substances.

A comparison of the above two methods has been made in an attempt to locate the sources of error in the method of Bessey *et al.*[1]

Comparison of the Two Techniques

In determining vitamin A levels in five serum samples (nos. 10, 41, 42, 47, and 49, in Table I) by the Bessey micromethod,[1] readings were made at 312, 328, and 336 mμ both before and after the destructive irradiation step. In other words, we actually determined points A, B, and C before irradiation and points D, E, and F after irradiation. This allowed us to compute vitamin A values both by the Bessey *et al.* method[1] and by the Morton and Stubbs procedure.[2,9] Each of these determinations was repeated with four or five different aliquots of the same serum so as to increase the precision of the mean values (Tables I and II).

In Table I, the results obtained by applying the Morton and Stubbs correction to these absorbance data are compared with the results obtained by the destructive irradiation procedure. The "true" vitamin A is given as a proportion of the total apparent vitamin A content of the sample; that is, as (B—E)/B. To convert these values to percentages they should be multiplied by 100.

Also listed in Table I are values from other reports in the literature. Several authors[1,3] give complete absorption curves for samples before and after irradiation. From these data one can make the above calculation and obtain values with a sufficient degree of accuracy for present purposes. The values attributed to

Bessey and co-workers in Table I were computed in this way from the curves given in their paper.[1] These curves contain a sufficient number of points to permit one to calculate "true" vitamin A by the method of Bessey *et al.*[1] and also by the method of Morton and Stubbs.[2,9] The values attributed to Little[3] in Table I are similarly estimated from the curves in his Figures 5 and 6. It is particularly to be noted that the effects demonstrated by our data can also be seen in those of Little[3] and Bessey.[1]

In Table I, the agreement between the two columns of values is not good. In other words, the agreement between the Bessey[1] and the Morton and Stubbs[2] methods is poor when applied to the same analytical data.

TABLE I

Fraction of Vitamin A in extract as Determined by Method of Morton and Stubbs[2] and by Destructive Irradiation Technique of Bessey *et al.*[1]

Sample	Serum vitamin A[a]	Proportion of "true" vitamin A in the extract as estimated by: Morton and Stubbs method	Destructive irradiation method	Difference between methods
	μg per 100 ml.			
Muscle[b]	—	0.91	0.53	0.38
"Whole" fish oil No. 3[b]	—	0.86	0.89	0.03
Unsap. fract. of No. 3[b]	—	0.74	1.00	0.26
"Whole" fish oil No. 4[b]	—	0.54	0.76	0.22
Serum No. 41	35	0.39	0.38	0.01
Unsap. fract. of No. 4[b]	—	0.38	0.93	0.58
Liver (II)[b]	—	0.24	1.00	0.76
Serum No. 47	16	0.24	0.23	0.18
Serum No. 42	19	0.24	0.32	0.00
Liver (III)[b]	—	0.18	0.54	0.36
Serum[c]	131	0.18	0.62	0.44
Serum No. 49	40	0.08	0.39	0.31
Serum No. 10	23	−0.74[d]	0.30	1.04

[a] As determined by the method of Bessey *et al.*[1]—values not available by this technique for the other samples.

[b] Values for these samples calculated from data in Figures 5 and 6 of Little.[3]

[c] Values for this sample calculated from data in Figure 2 of Bessey *et al.*[1]

[d] Inverted peak at 328 mμ.

Destruction of Non-vitamin A Substances by Ultraviolet Irradiation

In an attempt to understand the reasons for the above discrepancies, it seemed worthwhile to apply the equation of Morton and Stubbs[2] to our data in a different way. We obtained values for all six points in Figure 1. Points A, B, and C were determined prior to irradiation and D, E, and F after the ultraviolet irradiation which destroyed vitamin A. Thus far in the discussion, only four of the values (shown in Figure 1) have been used: B and E in the Bessey method and A, B, and C in the Morton and Stubbs method.

By making use of all six determined values we can study the nature of the material destroyed by ultraviolet irradiation, and thus test Demarest's second condition[5] directly. For this test we will apply the procedure of Morton and Stubbs[2,9] to the differences in absorbance readings obtained before and after irradiation (i.e., A−D, B−E, and C−F). If the conditions of Demarest hold, the resulting coefficients should be uniformly 1.00.* In other words, the absorbance values secured by the above subtractions should be the same as those for pure vitamin A. The results of this calculation indicate the degree to which the cross-hatched area in Figure 1 deviates from that for vitamin A.

Table II shows the results of the above calculations. Instead of a uniform set of 1.00 values, they actually range from −0.57 to +1.22. From this it would appear that the material destroyed during irradiation was not pure vitamin A, but ranged from −57 per cent to +122 per cent vitamin A. We interpret values which deviate from 1.00 as indicating that the ultraviolet irradiation destroyed non-vitamin A materials absorbing in the general range 312–336 mμ.

One thing appears certain. Demarest's

* This assumes compliance with the conditions of Demarest at 312 mμ and 336 mμ, as well as at 328 mμ. In case the degradation products of pure vitamin A have a small absorbance at 312 or 336 mμ, it would be appropriate to use a reference coefficient other than 1.00 (0.8 is closer to that observed in the data of Bessey *et al.*[1]). However, it is the constancy rather than the exact size of this coefficient which is material to the present argument.

TABLE II

Application of the Morton and Stubbs Method[a] to the Difference Curve[a] as a Means of Testing It for the Purity of the Vitamin A Destroyed

Sample	Proportion of "true" vitamin A in the material destroyed by ultraviolet irradiation[d]
Muscle[b]	−0.57[e]
"Whole" fish oil No. 3[b]	1.03
Unsap. fraction No. 3[b]	0.86
"Whole" fish oil No. 4[b]	0.89
Serum No. 41	1.02
Unsap. fraction No. 4[b]	0.49
Liver (II)[b]	0.24
Serum No. 47	0.56
Serum No. 42	1.22
Liver (III)[b]	0.38
Serum[c]	0.77
Serum No. 49	0.48
Serum No. 10	0.03

[a] Absorbancy difference at 312, 328, and 336 mμ resulting from destructive irradiation with ultraviolet light, i.e., (A − D), (B − E) and (C − F).

[b] Little[3] data.

[c] Bessey *et al.*[1] data.

[d] All values in this column should be 1.00 if nothing but vitamin A is changed by the destructive irradiation.

[e] Inverted peak.

second condition is not met. This is the case both in our data and in those of Bessey *et al.*[1] and Little.[3]

Estimation of Error

A quantitative evaluation of this method requires a consideration of a number of factors. The precision of Vitamin A analyses in several laboratories is given in Table III. The within-laboratory variation ranged from 2–24 per cent. Much of this difference in percentage variation is due to the differences in the average vitamin A concentrations of the samples used. Thus, when the results are expressed in terms of micrograms of vitamin A per 100 ml, the agreement between laboratories is considerably improved.

The data reported in the present study were obtained at one of the U. S. Public Health Service Nutrition Field units. The characteristic precision and the factors affecting precision of vitamin A determination by the Bessey *et al.*[1] method in this laboratory are

TABLE III

Variability in the Chemical and Spectrophotometric Determination of Vitamin A

Laboratory	Within laboratory standard deviation		Between laboratories standard deviation	
	as μg % vit. A	as % of mean	as μg % vit. A	as % of mean
Bessey *et al.*[1]	1	2	5[a]	8
U. S. Public Health Service	4	22	—	—
Sheffield	6[b]	24	3	11
Sheffield	5[c]	13	—	—
Little	—	5[d]	—	—
Clayton *et al.*[11]	11	21	16	30

[a] Between different methods within the same laboratory.

[b] Estimated from data in Protocol B.[10] Since this figure is calculated from an interaction term, it may be an overestimate.

[c] Estimated from Table 39.[10]

[d] Average of the deviations reported in Tables I, II, and III.[3]

defined in Table IV. The instrumental precision in this laboratory has been discussed previously.[12] The total standard deviation in the U. S. Public Health Service data is not as small as the within-laboratory precision quoted by Bessey *et al.*[1]; however, it is small

TABLE IV

Sources of Within-laboratory Error in U. S. Public Health Service Data[a]

	σ as %
Random manipulative and instrumental errors	0.5
Extraction errors resulting in:	
(a) Differences in shape of absorption curves of duplicate extracts of same serum	0.7
(b) Difference in average level of absorption (due to variations in total amount of material extracted, amount of turbidity present, etc.)	3.2
Irradiation errors due to differences in shapes of the absorption curves after irradiation	2.4
Total variation[b]	4.1

[a] See Table III.

[b] $\sigma_{total} = \sqrt{0.5^2 + 0.7^2 + 3.2^2 + 2.4^2} = 4.1\%$

when compared with the discrepancies noted in Tables I and II. To increase the precision of the data used in Tables I and II, all serum

samples were analyzed in at least quadruplicate. The certainty of our final conclusions is further increased by the finding of similar results in the data of others.[1,3]

As seen in the last columns of Table III, there is poorer agreement between the results obtained by different methods and different laboratories than is observed within a given laboratory using a single method. This is a frequent finding in collaborative assay work and may be due to the presence of unknown and uncontrolled factors in the method which nevertheless affect the analytical accuracy.

In Table I there was a 34 per cent average discrepancy between the two methods. The data of Table II deviated from 1.00 by 47 per cent on the average. These figures imply that the destructive irradiation procedure in the Bessey *et al.*[1] method is the source of an approximately 40 per cent error. This statement requires further clarification, however.

The difference between the two sets of analytical results in Table I may be due as much to errors in the Morton and Stubbs method[2] as to errors in the Bessey *et al.* method.[1] The negative value for serum No. 10 in Table I is an example of a failure on the part of the Morton and Stubbs procedure. In general, whenever the blank absorption curve (DEF) is non-linear, the Morton and Stubbs method may be in error. This becomes important only when the non-vitamin A absorbance is large with respect to the vitamin A absorbance (E approaches B). We may, therefore, expect the Morton and Stubbs method[2] to be no more reliable than the method of Bessey *et al.*[1] when applied to the determination of vitamin A in serum samples where there is little vitamin A and much interfering material.

The above difficulty is not present in quite the same way when the method of Morton and Stubbs[2] is applied to the study of absorption differences as reported in Table II. Here the latter technique serves as a useful and reliable test for compliance with Demarest's[5] conditions. If all three of his conditions are met, the area between ABC and DEF in Figure 1 must have the same shape as vitamin A. Thus, whenever the method of Bessey *et al.*[1] satisfies these conditions, all values in Table II should be 1.00. This generally was not true for our data or for that reported by others. The discrepancies were large. This fact casts doubt on the validity of the method of Bessey *et al.*[1] when applied to plasma samples.

The interpretation of the average deviation shown in Table II (47 per cent) may be open to some question (see page 411, footnote). If the shape of the absorption curve indicates that 47 per cent of the material destroyed as a result of ultraviolet irradiation was not vitamin A, does that necessarily mean that the 328 mμ absorbance is in error by that amount? Under such circumstances, the blank is usually large, and it would seem possible that there could be a larger (or smaller) change in blank at 312 mμ or 336 mμ than at 328 mμ as a result of the ultraviolet irradiation. If this were so, the 47 per cent average deviation might not be a true reflection of the error at 328 mμ.

A further problem is encountered as a result of the irregular distribution of the 47 per cent error. In some samples the error is very small. This is true for serum No. 41, where the results secured by the two methods agree very well (Table I). The results in Table II also suggest that very little if any non-vitamin A material in serum No. 41 was destroyed by ultraviolet irradiation. The great variations in the destruction of the non-vitamin A material by ultraviolet irradiation may depend upon some unknown characteristic of the sample. Instead of stating that the method is in error by a consistent factor of 47 per cent due to its failure to satisfy Demarest's second condition, it would seem more nearly correct to say that in the case of certain samples the method worked very well, while in other cases large errors were observed. Thus, any average error figure is dependent upon the sample involved.

Serum Vitamin A: Physiological Significance

Besides evaluating the accuracy of the analytical procedure proposed for the determination of serum vitamin A, it is essential to examine the physiological bases for any suggested interrelationships resulting therefrom. Several investigators[13,14] have proposed the

level of vitamin A in the blood as an index of nutritional status with respect to that vitamin. In order to evaluate the significance of the serum vitamin A levels, it will be necessary to accept the values that have been reported in the literature.

During prolonged experiments with vitamin A carried out with human beings,[10,15,16] the serum carotene levels decrease rapidly in the early part of vitamin A depletion, while the serum vitamin A levels are resistant to change. In the few cases in which a decrease in plasma vitamin A was actually found, it was only after other signs of acute deficiency had appeared.[10] It is further noted that serum vitamin A levels are highly characteristic of the individual,[10] which adds to the difficulty of interpretation.

Wald, Brouha, and Johnson[16] and Brenner and Roberts[15] noted a rapid decrease in serum carotenoids in their subjects maintained on a vitamin A-deficient diet for six months and seven and one-half months, respectively. Although there were no changes in the serum vitamin A levels, some of the subjects[16] showed signs of vitamin A deficiency. On the other hand, Control Subject SB[15] on 10,000 IU of vitamin A per day consistently maintained lower serum vitamin A levels than any of the deficient subjects maintained on a low vitamin A diet. In both experiments the serum carotenoids decreased early in depletion.

During World War II, a prolonged experiment on the vitamin A requirement of human adults was carried out at Sheffield, England.[10] Clinical signs of vitamin A deficiency were observed in a number of the subjects after they had been on the deficient diet for periods of eight months or more. Here, as in the previous reports, the plasma carotenoids decreased early in the depletion, while the vitamin A level showed practically no change.

Table XLIX in the report of the above study[10] shows that in most cases there was no significant difference between the plasma vitamin A levels of the deficient group and the normal control group, even after the deficient group had been on an approximately zero vitamin A intake for periods of over one year. It is also of interest that after the first month of the depletion there were no significant differences between the plasma carotenoid levels of the different individuals within each intake group, but in the case of the plasma vitamin A levels there were marked individual differences. The plasma vitamin A levels thus seem to be more characteristic of the individual and less characteristic of the vitamin A intake.

TABLE V

Average Plasma Levels in the Sheffield Study[10]

	Deficient subjects	Normal subjects	"Supercharged" subjects
	μg/100 ml	*μg/100 ml*	*μg/100 ml*
Vitamin A			
Mean	19	26	28
Range	7–40	11–44	9–50
Carotenoids			
Mean	13	77	[a]
Range	1–22	13–156	

[a] No comparable values are available in the Sheffield study.[10] The supplements given were in the form of pure vitamin A. Bessey and Lowry[13] suggest that in order to be indicative of an excellent dietary intake of vitamin A and carotene, the serum carotene levels should be 200 μg/100 ml or more.

Table V summarizes the findings for the various vitamin A intake groups. The values were obtained from the data of the Sheffield study as presented in their Protocol B.[10] The values for the "normal subjects" include the initial values of all subjects before starting the experiment, and the serum levels of the control subjects that were maintained on a normal vitamin A intake (5000 IU/day). The values for the "deficient subjects" include the values for the experimental subjects after they had been on the basal diet (about 65 IU vitamin A/day) for more than 12 months. The values for the "supercharged subjects" are the blood levels of subjects that had been on an intake of 25,000 IU (or more) of vitamin A per day for more than two months.

Only four of the sixteen deficient subjects showed a definite decrease in plasma vitamin A level.[10] Three of these, those who showed a marked impairment in dark adaptation,[10] reached average plasma vitamin A levels as low as 8–12 μg/100 ml. The day-to-day variation, however, was large in all of the plasma vitamin A values.

Further evidence concerning the physiological significance of plasma vitamin A levels is secured from a different type of data. One purpose of tests used in evaluating nutritional status is to estimate the reserve supplies of the nutrient in the body. For vitamin A, the primary storage site is the liver. There is evidence that in human beings the plasma level of vitamin A is not related to its concentration in the liver.[17-19]

A number of physiological factors are known to influence plasma vitamin A levels. An elevated body temperature, whether due to a disease process[20] or artificially produced,[21] results in a prompt and dramatic reduction in the plasma vitamin A level. Within a few days after the body temperature has returned to normal, the plasma vitamin A level increases without any dietary supplementation.[20,21] Gastrointestinal disturbances which hinder the absorption of fats may be associated with decreased levels of vitamin A in the plasma. These disturbances include celiac disease, sprue, carcinoma of the gastrointestinal tract, and liver disease.[19,21,22] In Bright's disease, the plasma vitamin A level may be very high, probably due to an inability of the liver to store the vitamin.[20] These aberrations in the plasma vitamin A levels have, however, been utilized in the diagnosis of some of the preceding conditions.

Discussion

A survey of a number of studies indicates that there is no relation between the serum vitamin A level and its dietary intake or its concentration in the liver. This conclusion agrees with an earlier suggestion.[23]

The method of Bessey *et al.*[1] that has been widely used in determining serum vitamin A has been shown to be subject to a number of errors, especially when applied to sera with low levels of vitamin A. In addition to analytical error, there are a number of normal and pathological sources of physiological variability. The total variation is the vector sum of the individual sources of variation. For a normal population, this can be represented by the following equation:

$$\sigma_{total} = \sqrt{\sigma_{mp}^2 + \sigma_c^2 + \sigma_d^2 + \sigma_i^2}$$

where:

σ_{mp} = the method precision as measured by the agreement between duplicate samples, observed within a given laboratory.

σ_c = the consistent analytical errors (over and above the variation between duplicate determinations) as measured by interlaboratory and intermethod discrepancies.

σ_d = the day-to-day variation in serum vitamin A values to be expected from an individual on constant intake (variation over and above the variations due to method error).

σ_i = consistent differences between the long-time averages of different individuals on the same vitamin A intake.

One may estimate that σ_{mp}, the precision of the micromethod of Bessey *et al.*,[1] is 5.4 μg/100 ml (mean of column 1 in Table III), while the consistent method error, σ_c, is 8.0 μg/100 ml (the mean of column 3 in Table III). A measure of the day-to-day variation in serum vitamin A values can be secured from the Sheffield study referred to above.[10] It was found that σ_d, the day-to-day variation in the group mean, was 5.4 μg/100 ml and σ_i, the average difference between the mean serum values of individuals on the same dietary intake, was 2.4 μg/100 ml. Combining these factors we secure:

$$\sigma_{total} = \sqrt{(5.4)^2 + (8.0)^2 + (5.4)^2 + (2.4)^2} = 11.3 \ \mu g/100 \text{ ml}$$

Within the data of any single laboratory the expected total variation would be:

$$\sigma_{total} = \sqrt{(5.4)^2 + (5.4)^2 + (2.4)^2} = 8.0 \ \mu g/100 \text{ ml}$$

In other words, the between-laboratory factor, σ_c, would be omitted from consideration. This 8.0 μg/100 ml variation, then, represents the variation one might expect in a set of blood samples from a group of individuals, all of whom had the same vitamin A intake for long periods of time, providing those samples were all analyzed in the same laboratory. In the interpretation of nutritional survey data it is often desirable to look at this statement from a slightly different point of view. Thus, if one were to find a population in which the variation in vitamin A value is approximately $\sigma = 8$

μg/100 ml, the conclusion follows that the individuals in that group are all on essentially the same vitamin A intake, insofar as this method is able to distinguish.

The standard deviation of the data reported by Bessey and Lowry[13] was about $\sigma = 9$ μg/100 ml. This agrees fairly well with the value calculated above. Here again we are led to the conclusion that serum vitamin A levels bear little or no relation to vitamin A intake or body stores. Unfortunately, no direct estimates of vitamin A intakes were reported in the survey.[13]

Summary

The micromethod of Bessey *et al.*[1] for serum vitamin A has been shown to be subject to a number of errors. The primary difficulty arises from the fact that ultraviolet light not only destroyed vitamin A but changed other substances that absorb at 328 mμ. The results secured by the above method did not agree with those from another spectrophotometric method. The differences were especially large for those serum samples containing low levels of vitamin A.

A survey of vitamin A studies with human beings shows that the serum vitamin A level is a characteristic of the individual to a greater extent than it is a reflection of the vitamin A intake. A number of physiological alterations result in large changes in the serum vitamin A levels. Furthermore, when the vitamin A intake is reduced, changes in serum vitamin A levels occur only after the deficiency state has become very prominent. Serum carotene levels are more responsive in adults to changes in the intake of vitamin A precursors (the primary source of vitamin A in adults).

The difficulties inherent in the various analytical methods for estimating vitamin A in the serum and the poor relationship between the level of that nutrient in the serum and nutritional status of the individual make the serum vitamin A level an unreliable measure of vitamin A nutritional status of either individuals or groups. The determination of the serum carotene level appears to be the procedure of choice for vitamin A studies with human beings.

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Disease: Deviation or Entity?

"'Disease' labels remain convenient symbols in those recurrent clinical patterns which are frequently isomorphic, as for example, in acromegaly, though they are less satisfactory where the variability of the clinical picture is much more marked, as, for example in rheumatoid arthritis. The dangers which the 'entity' concept carries are (i) that it promotes a 'penny-in-the-slot machine' approach to diagnosis by seeking for pathognomonic signs especially the short cuts of the laboratory or instrument; (ii) that it suggests that diagnosis is arrived at by comparing an unknown with a catalogue of knowns: the method of recognizing an elephant by having seen one before; (iii) that it reduces thought to a minimum; (iv) that it is of little help and may be positively misleading where the disease process varies significantly from the usual, and (v) that it leads to all those dangers associated with a label which Cowper implied when he wrote of those—'who to the fascination of a name, Surrender judgment, hoodwinked.'

"The second concept—deviation from the normal—interprets disease rationally in terms of anatomy and physiology. The simplest changes are *quantitative deviations from the normal*. . . . These simple quantitative deviations from the normal are, however, commonly combined in constantly recurring patterns (isomorphism); these we label 'syndromes.' Of these, three groups are clearly recognizable. The first is *anatomical*. . . . The second . . . are *physiological* (and here we include also *psychological*). . . . Thirdly, the syndrome might indicate *pathological* changes. . . .

"It is this concept which should dominate our teaching and our approach to medicine. In brief it may be stated thus: (*a*) disease indicates deviations from the normal—these are its symptoms and signs; (*b*) symptoms and signs are commonly found to recur in constant patterns; these are the 'syndromes' or 'symptom-complexes'; (*c*) these syndromes always indicate one or more of three aspects of disease: (1) its site, (2) associated functional disturbances, (3) causative factors in terms of (i) morbid anatomy, physiology and psychology, and (ii) etiology."
—Sir Henry Cohen. *Proceedings of the Royal Society of Medicine* 48: 155, 1955.

Endogenous Calcium in the Feces of Adult Man and the Amount of Calcium Absorbed from Food

By CONSTANCE L. BRINE, PH.D.* AND FRANCES A. JOHNSTON, PH.D†

AT PRESENT, no method is known for finding the amount of calcium absorbed from an entire diet, although methods for determining the amount of calcium absorbed from food added to a basal diet and from radioactive calcium salts are known and have been used. Feces contain so-called "endogenous" calcium, as well as calcium not absorbed from the food. If an estimate of endogenous calcium could be made, the amount of calcium unabsorbed and the amount absorbed from the whole diet could be calculated.

The endogenous calcium in the feces is made up mainly of calcium from digestive juices. After studying the literature, the authors[1] estimated that about 500 mg of calcium per day is secreted into the digestive tract. This is close to a similar estimate of 650 mg made by Stearns.[2] Only a part of this appears in the feces, because much of it is reabsorbed. The endogenous fraction of fecal calcium may also contain some calcium which has been secreted directly through the wall of the digestive tract. Recent research indicates that this amount is negligible.

The amount of endogenous calcium in the feces of human beings has been studied with the use of Ca^{45} by Blau *et al.*[3] They studied two subjects and concluded that one subject lost 91 mg and the other 117 mg per day of calcium in the feces from endogenous sources. There is no assurance, however, that these were normal values, since both subjects were suffering from bone disease.

The determination of endogenous calcium from the fecal calcium of subjects on a calcium-free diet is not feasible, since, even with the addition of a salt mixture and pure vitamins, no calcium-free diet has been devised which is palatable and adequate. The object of the present study was to find the amount of calcium in the feces at a zero intake without feeding a calcium-free diet. The plan was to find the calcium content of the feces at many different intakes of calcium, plot fecal calcium on calcium intake per day, and extrapolate the resulting curve to zero intake. Data for this are available in the many calcium balance studies already made.

AN ESTIMATE OF ENDOGENOUS CALCIUM IN THE FECES

The literature was reviewed for studies on adults in which values for the calcium content of the food and of the feces were reported. Only those experiments were included in which the experimental periods were of acceptable length. A period of six days was considered sufficient, provided the subject had been living on a diet the calcium content of which differed by no more than 200 mg from the experimental one. If an experiment did not fulfill the latter provision, the authors accepted the data if an additional three-day preliminary period for adjustment was employed. No studies on abnormal subjects were included. Those studies in which citrate, magnesium chloride, and sodium phosphate were fed, and in which there were large amounts of food containing phytates and oxalates, were omitted. Suitable data were collected from 51 reports published before January 1953.

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In some experiments the collection periods were much longer and the resulting values more characteristic than in others. For that reason, a weighting system was devised. Those experimental periods from 6 through 11 days in length were given a weighting of one; those from 12 through 17 days given two; and those from 18 through 23 days given three. Experiments which extended beyond 23 days on one level of intake were given no additional weighting because a higher weighting would allow one subject to affect the results too much. A single set of food and fecal values for one subject with a weighting of one is called a "subject-period," and 1226 of them were used. Some do not appear in Figure 1 and Table I because the intakes were above those plotted or listed.

For each 25-mg range of intake, the mean food and fecal values were found, and the fecal values were plotted against the intake values. A curve was fitted to the data by inspection and extrapolated to zero intake. At zero intake the amount in the feces was about 75 mg (Fig. 1). This, then, may be said to represent the endogenous calcium excretion. The value of 75 mg is not an exact one, but is probably not far from the true value for the mean for a large number of people. No doubt the value varies considerably from individual to individual.

The method of finding the endogenous calcium by extrapolating fecal values to zero intake is a valid one only if the amount of endogenous calcium in the feces is constant for adults. Studies on cattle[4] with radioactive calcium show that the amount of endogenous calcium does not change during the years of maturity, although it increases in old age, and that it is little affected by the diet[5] being consumed at the time of the experiment. Growing rats[6] which had been living on a high calcium diet long enough to build up a large reserve excreted more endogenous calcium than those animals with poor stores when both were given radioactive calcium. Whether this would apply to human adults is not known. Even if the stores of adults affect the endogenous calcium, this may not invalidate the method of using a curve extrapolated to zero, provided the stores of the subjects do not vary in relationship to the calcium content of the experimental diet, but in a random fashion. If the stores affect the amount of endogenous calcium, the deviation from the mean of 75 mg for individuals would be much wider than if that variable were not playing a part.

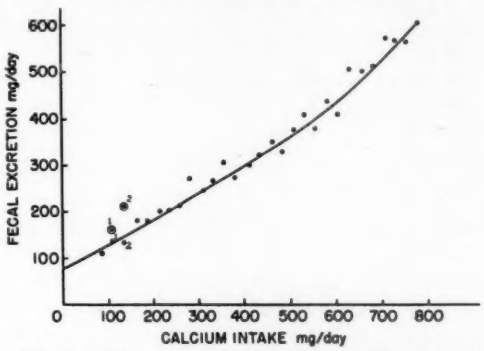

Fig. 1. The amount of calcium in the feces at each 25-mg range of food intake for 986 subject-periods.
[1] The circled dot is for a mean of 31 subject-periods falling into the 100–125 mg range of intake. The plain dot is for the mean with 3 subject-periods omitted because fecal values were unusually high.
[2] The circled dot is for a mean of 17 subject-periods falling into the 125–150 mg range of intake. The plain dot is for the mean with 2 subject-periods omitted because fecal values were unusually high.

An Estimate of the Amount of Calcium Absorbed

The object of finding the amount of endogenous calcium in the feces was to use it in calculating the amount that is absorbed. The subject-periods were arranged in classes of 200 mg, ranging from zero intake to 1200 mg per day (Table I). The mean percentage absorbed when calculated from the difference between food and fecal values was determined; then this figure was corrected for endogenous fecal calcium, using the value of 75 mg. When this was done, the amount absorbed at intakes of 400 to 599 mg was 43 per cent. Beyond that point, the amount fell gradually as the intake increased: from an intake of 600 to 999 mg, 34 to 35 per cent of the calcium was absorbed, and from 1000 to 1199 mg, the amount absorbed dropped to a mean of 28 per cent. The largest number of subjects fell in the 400- to 599-mg intake level and, there-

TABLE I

The Amount of Calcium Absorbed at All Levels of Intake before and after Correction for 75 mg of Endogenous Calcium in the Feces

Intake	No. of subject-periods	% Absorbed (uncorrected)	% Absorbed (corrected)
mg/day			
0–199	105*	−18	38
200–399	267	14	42
400–599	359	28	43
600–799	250	24	35
800–999	94	24	34
1000–1199	81	21	28

* Five values for subjects with abnormally high losses of calcium have been omitted.

fore, the value for that range may be the best estimate.

The swing upward in the amount of calcium in the feces (Fig. 1) beginning at an intake of about 600 mg may be due either to a decrease in the percentage of calcium absorbed, or to an increase in the amount of endogenous calcium, or to both. In most cases, the workers who carried out the studies have made no report concerning the previous diet of the subjects; therefore, there is no way of knowing whether the subjects on the experimental diets in which high intakes were used had better stores than those placed on low intakes. The authors are inclined to believe (on the theory that subjects with high stores were randomly distributed) that this upward swing was due mainly to a decrease in the amount absorbed.

The values for the percentage of calcium absorbed agree fairly well with those found when a test-food is added to a basal diet. Ten experiments of this type were compiled, including 88 subjects whose total intake, after the addition of the test-food, lay between 400 and 1000 mg per day. The mean percentage of calcium absorbed was 34. The values found in the present study agree also with those found by Geissberger,[7] who used radioactive calcium salts. He fed three adults 1.0 g of calcium gluconate containing Ca^{45}. After four days, two of them had excreted 65 per cent of the Ca^{45} by way of the feces. Apparently they had absorbed about 35 per cent. No correction was made for Ca^{45} absorbed, secreted in digestive juices and not reabsorbed.

In 1939, Mitchell and Curzon[8] calculated a regression equation from a compilation of values taken from calcium balance studies in the literature, but for a different purpose. Their values cannot, therefore, be compared to the ones found in this study.

Summary

The amount of calcium in the feces when the food contains none was found by plotting the fecal calcium of subjects reported in 51 studies in the literature against dietary calcium and extrapolating to zero. The feces were estimated to contain 75 mg per person per day of endogenous calcium. This figure was used in calculating the percentage of calcium absorbed in a compilation of reported studies which supplied intake and fecal values for calcium. The mean percentage absorbed on intakes from 400 to 599 mg per day was 43; on intakes from 600 to 999 mg, it was 34 to 35; and on intakes from 1000 to 1199 mg, it was 28.

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Editorial

Do Our Patients Understand Us?

Every physician has faced the frustrating experience of seeing his best professional advice ignored. This is perhaps especially true in nutritional work, where "lapses" or non-co-operation are almost par for the course among many patients with obesity, peptic ulcer, gallbladder disease, or hypertension. The accumulated knowledge of countless investigators is condensed into our recommendations and prohibitions; yet how frequently do we find obvious and flagrant violations of these well-meaning admonitions. Why?

No doubt many reasons are involved, many derived from deep-seated psychologic or social roots.[1] But one cause (recognized in theory but frequently forgotten in practice) is the clinician's fault. *Do our patients understand us?*

A recent study by a nutritionist on the Florida State Board of Health[2] indicates a serious break in the chain of communication from Science through Physician to Patient. A list of 20 commonly used words was presented to 100 women attending antepartum clinics in various parts of the state. This group consisted of 95 colored and 5 white women aged 15 to 46, with an average age of 26 years. Only 80 per cent apparently read occasionally or regularly. Half had elementary school education, and half had some high school experience. About half were from rural areas. After a good rapport was established with the patient, the meaning of the words was asked, and the word was used in a sentence. Any reasonable definition was accepted and no time limit was imposed. Here are the startling results.

While 90 per cent of the women had some understanding of such words as *pregnancy, constipation, margarine, starches,* and *diet,* fewer than 20 per cent understood *anemia, rickets, hemoglobin, enriched,* or *protein.*

Somewhat more than half knew the meaning of *well nourished, vitamins, dried milk, yellow vegetables, lean meat,* and *egg yolk.* But less than half could explain *nutrition, maternity, citrus fruits,* or *balanced diet.*

On the average, only 12 words of the 20 were understood. Not one patient understood every word. Only 17 patients knew the meaning of 16 or more terms. Those with some high school training could comprehend an average of 13.4 words, those with elementary schooling only 10.4. Curiously, women from homes with radios did not score significantly different results from those without that modern propaganda and educational device.

Among the interesting sidelights to this survey were: one out of four did not know which part of the egg is called "yolk"; "vitamins" often meant some kind of pills—not related to food or nutrition; anemia was often confused with enema.

The significance of the implications of this study are all too clear. Although the group questioned may not be typical of the reader's practice, and the technique of the study not completely acceptable to the more chi-square minded, nevertheless, a great service has been done in reminding clinicians that the patient may not fully appreciate what is being said.

There is a need for a constant awareness of the terminology used by physician, nurse, dietitian, or any health worker. Literature

such as diet lists should perhaps be written on an even simpler level and, in addition, verbally explained at the time of distribution. In fact, because many patients believe they understand but actually have an erroneous conception of certain terms, or nod knowingly rather than admit ignorance, it is our responsibility to make doubly sure that we and our patients truly speak the same language. We can well wonder how often failure to follow dietary instruction stems from lack of understanding of the advice given. Let us then add an aphorism—Physician, explain thyself!

—S. O. WAIFE, M.D.

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In our Next Issue

NUTRITION IN SURGERY—A SYMPOSIUM

I. S. Ravdin, M.D., Guest Editor

Principles of Surgical Nutrition
ROGER D. WILLIAMS AND ROBERT M. ZOLLINGER

Nutritional Evaluation during the Altered Physiological State after Injury
JOHN M. HOWARD

Nutrition and Wound Healing
PAUL MECRAY, JR.

Supplementation of Protein and Caloric Needs in the Surgical Patient
HAROLD G. BARKER

The Judicious Use of Saline and Glucose Solutions before and after Surgical Operations
JAMES D. HARDY

Jejunal Feeding
THOMAS E. MACHELLA AND ROBERT G. RAVDIN

Some Aspects of Nutrition in the Pediatric Surgical Patient
HAROLD A. ZINTEL

Changing Concepts in the Surgery of the Diabetic Patient
WALTER M. BORTZ, II AND EDWARD L. BORTZ

Diet Therapy

DIETARY PROCEDURES IN THE METABOLIC UNIT

By LEILA F. PRESSON, M. Sc.*

IN THE PAST few years there has been an increased need for dietitians trained in metabolic procedures. The principles of the different types of diets and some of the metabolic procedures have been described.[1-5] This article presents some of the current practices and describes the metabolic kitchen at the Massachusetts General Hospital.

THE SELECTION AND PURCHASE OF FOODS

Bread. Only white bread, either salted or salt-free, is used. This bread is baked daily in the hospital from standardized recipes. Brown bread is not baked in this hospital but is purchased, and presents a problem to the dietitian who is not directly in charge of buying. The salt-free bread is low in calcium (about the same as the original low calcium bread).[3] It can be used for the low calcium diets, and is especially helpful if the patient eats several slices of bread daily.

Butter. Only salt-free butter is used. The same brand is used throughout the year and is bought by the case.

Cereals. The same brands of cereal, macaroni, and rice are used throughout the year. Prepared cereals include cornflakes, Rice Krispies, Shredded Wheat, and Puffed Rice. Oatmeal and farina are most popular among the cooked cereals. Macaroni, rice, and farina may be bought in bulk in quantities sufficient to last for a year.

Eggs. Fresh eggs are used and are probably more variable than most foods used. The only measure taken to keep eggs constant is to buy the same grade. Even then there is often a variability of 10 grams in the weight of the individual eggs.

Fruits. When choosing fruits to be used on a metabolic ward, the dietitian must select ones which will have the least chemical variability and the greatest palatability. Canned fruits which may be purchased from the same lot in quantities sufficient for a study are less variable than fresh fruits which would be purchased several different times for a study of 3–4 months. Of the canned fruits, pears, peaches, applesauce, pineapple, and apricot halves are most popular. The fruits are canned with sugar. Berries, and fruits with pits such as cherries and plums do not look appetizing after they are pitted and drained. Berries with seeds present the disadvantage of plugging pipettes when stools are being analyzed. Bananas and fresh apples are not often used, since it is practically impossible to obtain them from the same source during a long balance study. The amount of canned fruit which will be needed in one year is estimated and ordered at the beginning of the year from a company which will fill the order from the same lot of canned goods. The buyer must be assured that all cans bearing the same lot number represent fruit harvested and processed at the same time of the year.

Fruit Juice. Canned unsweetened grapefruit, grape, and pineapple juice are selected in the same manner as the canned fruits. The only fresh juice used is lemon juice. There is more variance in fresh lemon juice than in the canned juices, but much smaller quantities are used in one diet. Frozen orange juice of the same brand throughout the years is used, and

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it is bought by the case. One case of 32-ounce cans will last from six weeks to two months, depending on the number of patients.

Meat. Long-term studies limit the types of meat which can be used on constant diets. The meat must be free of bone, gristle, and fat. Breast of fowl, tenderloin steaks, and ground beef meet all of these requirements. The fowl breasts are bought from the same dealer each week. Breasts are used in order to have only white meat, since white meat and dark meat are different in mineral content. Whole tenderloins are bought and trimmed by the butcher. One tenderloin will give fifteen to twenty 4-ounce steaks. Hamburger from the top of the round is used, and is probably the most constant of the three meats. Enough hamburger for a study of any length can be obtained at one time. There is no danger of a patient getting a piece of gristle in a hamburger pattie. This may happen occasionally with steaks.

Milk. Homogenized milk or skim milk from the same company are used. Both of these eliminate the difficulty encountered when cream is unevenly distributed in milk. Cases of evaporated milk from the same lot may be used, but most patients refuse milk rather than drink evaporated milk.

Vegetables. The only fresh vegetable used is the Idaho potato. A bushel at a time is set aside for the research ward, and this lasts from six weeks to two months depending on the number of patients. Vegetables other than potatoes are canned (with salt unless the diet is restricted in salt). Peas, string beans, tomatoes, whole kernel corn, beets, and carrots, are most popular. The vegetables are bought at the beginning of the year from the same lot. (Corn is not used on diets for patients whose stools are to be analyzed. It often shows up in the stool as whole kernels and interferes with pipetting.)

Miscellaneous. Protenum® and other high protein supplements, homogenized peanut butter, catsup, jello, saltines, graham crackers, vanilla wafers, jelly, honey, sugar, etc. have been used on constant diets. An effort is made to use the same brands of these products and to obtain a supply which will last through one study.

Salt. Chemically pure salt is secured from the pharmacy.

Cooking and Weighing Procedures to Keep the Food Constant

Bread. The total for the day is weighed the previous day, wrapped in wax paper which is carefully labeled with the patient's name, the weight, and the kind of bread (salt-free or regular). Graham crackers and saltines are weighed and given to the patient in waxed sandwich bags.

Butter. The salt-free butter is weighed and the total must be used by the end of the day. Greater accuracy and even distribution can be assured if the dietitian butters bread and toast for the patient.

Cereals. The weighed cereal is placed with a measured amount of water into a cereal bowl, cooked in a steamer, and served in the same bowl. The amount of water is adapted to the patient's taste and is constant daily. Macaroni and rice are cooked in the same manner. This method is more accurate than cooking the cereal in a double boiler and weighing the cooked cereal. Proportions of water commonly used for cereals are:

8 to 10 cc water per gram of farina
5 to 6 cc water per gram of oatmeal
4 cc water per gram of rice
4 cc water per gram of macaroni.

In Boston the tap water is sufficiently mineral-free for use in these studies.

Eggs. Eggs are beaten, weighed into casserole dishes, scrambled in individual frying pans in a portion of the patient's butter, and served in the casserole dish.

Fruit. Canned fruits are drained on paper towels, and weighed into sauce dishes from which they are served. They are covered with wax paper, which is marked with the patient's name and the weight of the fruit.

Fruit Juices and Milk. These are weighed into glasses which are marked with the weight, kind of juice, patient's name, and the time it is to be served. Each glass is covered with a

bottle cap between two layers of wax paper, which are fastened with a rubber band. The bottle cap contains the same information as the glass. This method gives two checks on labeling. It has proved more satisfactory than the use of large soufflé cups inverted as covers.

Meat. Chicken breasts are thawed, cooked in the steamer, and freed of skin. The cold chicken is weighed and reheated in a casserole dish before serving. Daily portions of hamburger and steak are weighed at the beginning of the study and frozen. One is removed from the freezer daily and cooked in a small amount of the patient's butter. Individual frying pans are used and are thoroughly scraped with a flexible spatula to remove all meat particles and gravy.

Sugar. The total sugar to be used each day is weighed into glass creamers which are labeled with the weight and the patient's name.

Salt. The total salt to be used each day is weighed into flat white envelopes (Bescup drinking cups) which are fastened with a paper clip. The patient must be instructed how to pour from the envelope. Salt shakers lose salt between the shaker and the cap. The salt is dried for 48 hours in an electric drying oven, and is then stored in a covered flask to keep it free from moisture. (Salt is weighed on a balance accurate to 0.01 grams.)

Vegetables. The canned vegetables, with the exception of tomatoes, are drained on paper towels placed on trays. These are weighed directly into casserole dishes with lids that fit over the dish. A hamburger pattie paper with the weight of the vegetable, the patient's name, and the meal, is placed between the dish and the cover. The paper serves the dual purpose of marking the dish and preventing the collection of moisture in the dish. The casserole dishes are heated in the steamer (not pressurized). The vegetable may be served in the dish in which it is heated. Potatoes are steamed, cooled, weighed, and reheated.

Miscellaneous. Honey, jelly, and catsup are weighed in glass creamers or in small waxed cups with smooth sides. Jello is made by placing the weighed amount of jello powder in a casserole dish, adding a measured amount of warm water, and allowing the mixture to gel. It is then served in the same dish. Lemonade is made by weighing the lemon juice into the glass, adding weighed sugar, and then adding the water, which has been measured in a graduate cylinder. (It is not stirred until just before serving, and the spoon is left in the lemonade for the patient to lick.) Eggnogs are made by weighing milk, adding weighed beaten eggs, and weighed sugar.

Analysis of Diet

The diet is routinely analyzed once or twice during a study. More analyses may be necessary if the food is changed (e.g. a new lot of steaks) during the study. A one-quarter aliquot of the diet is sent to the laboratory, and includes everything except sugar, sodium chloride, and salt-free butter. Cereals, rice, or oatmeal are cooked before being added to the aliquot for analysis. Two or three one-quarter aliquots of meat are weighed at the beginning of the study when the meat is weighed into individual portions. When the aliquot is being prepared it is necessary only to remove one aliquot of meat from the freezer and add it to the mixture. Salt-free butter may be analyzed separately or assumed to be mineral-free fat.

Procedure for Starting a Diet

The choice of a patient for a metabolic study is made by the physician. The patient on a metabolic study should understand that he is to be on a constant diet, since this may be the most difficult part of the study for him. This information should be made available to the patient before he agrees to come to the research ward.

Using only one menu for an entire study has the disadvantage of being more monotonous for the patient, but has several advantages. The patient can have a trial period for the diet, whereas it would be impractical to have trial periods for two or three diets which are changed every third or fourth day. Much less time is needed for the dietitian to figure

one diet, and there are fewer possibilities for errors when changes are not made every three days. Also, only one diet need be analyzed by the laboratory. The foods used on a constant diet are somewhat limited, and the patient usually chooses the food he likes best; therefore, the few changes which can be made do not seem to justify the extra work for all the people involved.

When the patient enters the ward, the dietitian explains the constant diet and helps the subject choose a menu for trial according to the diet prescription ordered by the physician. Three to five days are needed to adjust the diet for most patients; however, one day of trial has been used if the study is as short as two weeks. The three trial days enable the patient to try different foods and become accustomed to eating everything given to him. During this time the dietitian should follow the weight chart to see if the number of calories in the diet are sufficient to maintain a constant body weight. The dietitian should have all the food needed by the end of the trial period.

After the patient has been placed on a constant diet, no changes are made in the diet without the physician's orders. If the patient is too ill to eat at some meal and the food cannot be eaten later, the food is saved for laboratory analysis. Birthdays and holidays will be difficult for some patients, since little can be done to celebrate except to use holiday tray covers and napkins. The only allowable variations in constant diets are simple ones, such as having eggs scrambled one day and in an eggnog another day. Dinners and suppers may be reversed, or individual foods changed from one meal to another, but this accomplishes little.

Patients tend to choose fewer calories than they have been eating outside the research ward. There are possibly three reasons for this—the fear of having more food than one can eat after the study has been in progress a week or two, the tendency to want less food if the same food is to be eaten every day, and the lower caloric value of the individual foods offered on a constant diet. The latter is particularly true of desserts if the patient has been accustomed to eating cakes, pastries, ice cream, etc.

Salt is the only condiment normally used for constant diets. Mixtures of foods are discouraged because of the difficulty in calculating replacements in case of spilling, and because of the time required to make individual mixtures for several metabolic studies.

Constant diets are lower than normal diets in fiber content and are often constipating to the patient. The food is bland and simple. A patient who has been on a diet for a month or two needs to "go easy" on seasonings, fried foods, etc., for the first few days after the study has been completed. Too often the patient overeats the first day and feels uncomfortable.

The Calculation of Special Diets

Dietitians with no previous experience in metabolic work may be unfamiliar with the calculation of diets requiring the balancing of milliequivalents. One of the most difficult to calculate is the neutral ash, 150-mg calcium diet, since the acid and base must be balanced, the calcium kept low, and adequate amounts of other nutrients supplied. If the patient does not like foods low in calcium, or if he chooses foods with an excess of acid ash or basic ash, the diet may present a problem. If a patient chooses foods higher in calcium, portions will be restricted and calories will be low unless the diet is supplemented with extra amounts of food such as butterfat, honey, jelly, and sugar. This may be unpleasant for the patient. If a patient chooses small servings of foods forming acid ash, foods forming basic ash must also be restricted.

Special Equipment Used in the Metabolic Kitchen

1. Torsion Balance—accurate to 0.1 gram. Several types are available; the type which will allow two dishes to be balanced simultaneously is more desirable. Information may be obtained from Torsion Balance Company, 92 Reade Street, New York City.

2. Trip Balance. This scale is not as accurate as the above for less than a gram, since the pointer cannot be read between gram

TABLE I.

Example of a 150-mg Calcium, Neutral Ash Diet

Amt.	Food	Acid	Base	CHO	Pro	Fat	Ca	Mg	K	Na	P	Cl	S
g		mEq	mEq	g	g	g	g	g	g	g	g	g	g
100	Grapefruit juice	—	4.1	11.1	0.4	0.1	0.010	0.008	0.139	0.005	0.017	0.002	0.005
20	Farina (uncooked)	2.0	—	15.3	2.2	0.3	0.004	0.005	0.024	0.013	0.025	0.015	0.031
90	Bread	5.8	—	47.7	8.1	1.1	0.027	0.027	0.097	0.401	0.087	0.558	0.048
70	Salt-free butter	0.2	—	—	0.7	59.8	0.010	trace	0.009	0.004	0.011	0.009	0.007
120	Hamburger	19.8	—	—	24.4	9.5	0.015	0.029	0.405	0.101	0.245	0.091	0.276
140	Tomato	—	7.8	3.9	1.0	0.2	0.015	0.017	0.373	0.025	0.037	0.040+	0.019
20	Rice (uncooked)	1.7	—	15.5	1.4	0.1	0.002	0.007	0.014	0.005	0.020	0.008	0.023
100	Applesauce	—	3.4	19.7	0.2	0.1	0.007	0.006	0.116	0.010	0.011	0.004	0.001
60	Chicken	7.0	—	—	15.3	1.5	0.009	0.016	0.223	0.054	0.130	0.047	0.155
100	Potato	—	8.9	19.1	2.0	0.1	0.011	0.027	0.496	0.024	0.056	0.035	0.029
60	Peas	—	0.5	5.8	2.1	0.1	0.013	0.016	0.171	0.162	0.073	0.019+	0.033
100	Pears	—	3.7	18.4	0.2	0.1	0.013	0.009	0.129	0.008	0.016	0.004	0.007
50	Lemon juice	—	1.9	4.2	0.5	0.2	0.011	0.005	0.074	0.006	0.006	0.002	0.004
100	Pineapple juice	—	6.1	13.0	0.3	0.1	0.008	0.010	0.270	0.016	0.011	0.051	0.009
60	Sugar	—	—	60.0	—	—	—	—	—	—	—	—	—
20	Grape jelly	—	—	13.0	—	—	0.002	—	—	—	—	—	—
Totals		36.5	36.4	236.7	58.8	73.3	0.157	0.182	2.540	0.834	0.745	0.885+	0.647

Note: The diet is considered essentially neutral if the milliequivalents agree within 0.5. Amounts of minerals are generally expressed in milligrams and milliequivalents.

The following formula may be used for calculating milliequivalents:

$$\frac{\text{milligrams}}{\text{atomic weight}} \times \text{valence} = \text{milliequivalents}$$

$$\frac{\text{e.g. 157 milligrams calcium}}{40} \times 2 = 7.85 \text{ milliequivalents}$$

or the following factors may be used:

Mineral	Atomic weight	Valence	Conversion factor
Calcium	40.07	2	0.05
Magnesium	24.32	2	0.0822
Potassium	39.10	1	0.02557
Sodium	23.00	1	0.0435
Phosphorus	31.04	2	0.06443
Chlorine	35.46	1	0.0282
Sulfur	32.06	2	0.0624

e.g. 157 milligrams calcium x 0.05 = 7.85 milliequivalents

marks. These balances must be checked often. Obtainable from Hanson Scale Company, Northbrook, Illinois.

3. A balance accurate to 0.01 gram is useful for salt.

Note: All balances should be checked routinely each year or more often if necessary.

4. Graduate Cylinders. 250-cc volumes are useful for measuring tea and coffee. Cylinders of less volume may be used for measuring fluids returned on trays of patients who may not be on complete metabolic studies.

5. Flexible spatulas. Stainless steel spatulas are used instead of knives on the trays. The patients can clean the plates with these spatulas and they are necessary for scraping the individual frying pans. Suitable flexible spatulas may be obtained from The McDonald Company, 23 Lansdowne Street, Boston, Massachusetts.

6. Casserole Dishes. Individual Hall china casserole dishes with lids fitting over the casserole rather than inside the casserole, to prevent collection of moisture in the dishes.

7. Frying Pans. Individual size frying pans are used. Frying pans may be obtained from the McDonald Company (above).

8. Steamer. A steamer is not found in some units, but they are quite helpful in heating foods and in cooking many foods. The steamer is not pressurized. It was obtained from Cleveland Range Company, Cleveland, Ohio. This steamer is small enough to set on a regular counter workspace rather than on the floor.

9. Freezer. At least two cubic feet of freezer space is needed for each person on a metabolic study.

10. Dripolator. Drip coffee is more constant than percolator coffee when the grounds are weighed and the water is measured. It is impossible to have coffee made exactly the same way each day in a percolator, and if the patient drank much coffee, this could possibly make a difference in studies closely controlled in potassium.

Summary: The present dietary procedures and a description of the metabolic kitchen at Massachusetts General Hospital have been presented.

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Nutrition News

☆ News of activities in the field of clinical nutrition should be submitted to the Editorial Office of the JOURNAL.

Arteriosclerosis Meeting

The Ninth Annual Meeting of the American Society for the Study of Arteriosclerosis will be held on Sunday to Monday, November 6 and 7, 1955, at the Sheraton Hotel, Chicago, Ill. The program of the meeting and abstracts of the 69 papers read will be published in the October issue of *Circulation*.

National Vitamin Foundation Grants

Sixteen new grants, totaling $70,766.00, have been extended to American universities and medical centers by the National Vitamin Foundation, Inc. Effective July 1, 1955, the awards are the latest in the Foundation's series of semi-annual grants-in-aid for clinical and laboratory research. Among the recipients of the new grants are:

Dr. Bacon F. Chow (Johns Hopkins University) for studies of vitamin B_{12} and aging.

Dr. George R. Cowgill (Yale University) for studies of the physiology and biochemistry of pantothenic acid deficiency.

Dr. Albert B. Eisenstein (Washington University of St. Louis) for studies of the relationship of pantothenic acid to adrenocortical hormone secretion.

Dr. George J. Gabuzda (Western Reserve University) for studies of the metabolism and interrelationship of folic acid, citrovorum factor, and ascorbic acid.

Dr. Irving Graef (4th Medical Division [New York University] Bellevue Hospital) for studies of the effect of pantothenic acid deficiency in various disease states.

Dr. G. Watson James, III (Medical College of Virginia) for studies of vitamin B_{12} growth activity of *P. stipitata* in human leukemia.

Dr. Albert L. Lehninger (Johns Hopkins University) for studies of the enzymatic mechanism of ascorbic acid biosynthesis in animal tissues.

Dr. Herman C. Lichstein (University of Minnesota) for studies of the use of compounds structurally related to biotin for the study of the mechanism of action of biotin.

Dr. A. Leonard Luhby (New York Medical College) for studies of the evaluation of urinary formamido-L-glutamic acid excretion as a clinical test for folic acid undernutrition.

Dr. O. Neal Miller (Tulane University of Louisiana) for studies on the metabolism of vitamin B_{12} in man with special reference to interactions among serum proteins, intrinsic factor, and the vitamin.

Dr. Theodore F. Zucker (Columbia University) for studies of pantothenic acid deficiency and the status of acetylcholine in intestinal tissue.

Nutrition Briefs

CURRENT OBSERVATIONS OF CLINICAL INTEREST

IN PATIENTS with hepatic cirrhosis there is a higher plasma level and urinary excretion of cystine. In general, the higher plasma levels occurred in the patients with the more severe liver damage. Cystine metabolism appears to be disturbed early in liver disease.

J. M. Walshe and B. Senior. *J. Clin. Investigation* 34: 302, 1955.

DIETARY fat supplies fatty acids necessary for cholesterol esterification and stimulates bile flow necessary for cholesterol esterase activity.

L. Swell, D. F. Flick, H. Field, Jr., and C. R. Treadwell. *Am. J. Physiology* 180: 124, 1955.

FED FREE cholesterol produced greater elevations in blood cholesterol levels than fed cholesterol esters.

L. Swell, T. A. Boiter, H. Field, Jr., and C. R. Treadwell. *Am. J. Physiology* 180: 129, 1955.

ADMINISTRATION of salicylates or ACTH does not appear to deplete vitamin C stores, under the conditions reported. Children receiving salicylates for rheumatic fever may not need excess vitamin C.

A. A. Albanese, R. A. Higgons, W. A. Avery, and R. Dilallo. *New York State J. M.* 55: 1167, 1955.

SIGNIFICANT differences were found in the serum concentration of fatty acids among well nourished and malnourished children.

A. E. Hansen. *Tenth M & R Pediatric Research Conference*, 1954, p. 51.

A DIETARY survey on 7,800 Wisconsin school children revealed that 10 to 20 per cent drank little or no milk, less than half ate an "adequate" amount of citrus fruit, and less than one-fourth had a daily serving of green or yellow vegetables. Excessive consumption of concentrated sweets was noted, while a "good" breakfast was found only among 10 to 60 per cent of children in different areas.

L. K. Billington. *Wisconsin M. J.* 54: 312, 1955.

THE MINIMAL human requirement for L-lysine is from 0.4 to 0.8 g/day. For various reasons, a safe intake is 1.6 g/day. Little if any D-lysine is used by man.

W. C. Rose, A. Borman, M. J. Coon, and G. F. Lambert. *J. Biol. Chem.* 214: 579, 1955.

WEIGHT gain was significantly greater among Navy recruits receiving Aureomycin and penicillin daily as compared to control subjects, according to this carefully conducted study.

T. H. Haight and W. E. Pierce. *J. Nutrition* 56: 151, 1955.

IN SPITE of obvious deficiencies in protein and certain vitamins, the incidence of dental caries was lower among poorly nourished school children in Guatemala than among United States Children. The very low content of refined carbohydrate in the diet is given as a possible explanation.

A. Hurtarte E., and N. S. Scrimshaw. *J. Dental Res.* 34: 390, 1955.

RECENT ADVANCES IN EXPERIMENTAL NUTRITION

THE hyperlipemia of experimental nephrosis is a transport lipidemia, i.e., mobilization of fat and cholesterol from storage sites, in response to a need for fat as a source of energy.

J. B. Marsh and D. L. Drabkin. *J. Biol. Chem.* 212: 633, 1955.

IRON was deposited in interstitial cells of testes after large doses of an iron-dextran complex were administered intravenously to rats.

J. A. Nissim. *Lancet* 1: 701, 1955.

OVERHEATED fat is said to contain a low-grade carcinogen which is active on subcutaneous administration to rats. Some people can ingest foods and drinks hot enough to injure the stomach wall. These factors may play a role in human gastric cancer.

A. C. Ivy. *Gastroenterology* 28: 345, 1955.

A STATISTICALLY significant lowering of the voluntary consumption of alcohol was produced when glutamine (glutamic acid plus ammonia) was fed orally to rats.

L. L. Rogers, R. B. Pelton, and R. J. Williams. *J. Biol. Chem.* 214: 503, 1955.

Reviews of Recent Books

The Vitamins: Chemistry, Physiology, Pathology, edited by W. H. Sebrell, Jr., and Robert S. Harris, Academic Press Inc., New York, 1954, **Vol. II,** pp. 766, **$16.50, Vol. III,** pp. 665, **$15.00.**

These two volumes complete the three-volume treatise on the chemistry, physiology, and pathology of the known vitamin factors which for many years to come will be the best source-book of factual information for all workers in the field. Written by numerous authorities and fully documented, these encyclopedia-like reference books seem to cover every significant observation dealing with the vitamins which have been made in the "basic sciences." Clinical matters receive rather scant treatment, but these books make no attempt to cover this aspect completely.

There is an ample subject and author index, and innumerable references which fully document the treasury of experimental work in this field. Anyone engaged in research with a particular vitamin substance will need to refer to this work for complete coverage. He will find these reviews entirely satisfactory.

Volume II deals with choline, inositol, vitamins D, K, niacin, pantothenic acid, and essential fatty acids. Volume III deals with pyridoxine, riboflavin, thiamine, tocopherols, p-aminobenzoic acid, pteroylglutamic acid, and new and unidentified growth factors. The authors and editors are to be congratulated on performing a difficult task in an admirable manner.

S.O.W.

Casimir Funk. Pioneer in Vitamins and Hormones, by Benjamin Harrow, Dodd, Mead & Co., New York, 1955, pp. 209, $4.00.

The story of the Polish chemist, Casimir Funk, who has been living in this country since 1915, has certain interesting elements. One of the early workers in nutrition and modern biochemistry, he is remembered for a number of contributions, including the coining of the word "Vitamine" in 1911.

Nevertheless, a very one-sided portrait is painted by his old friend and co-worker, Dr. Harrow. The book is a typical panegyric—from the preface in which "Funk represents one of the original minds of our time" . . . . "a pioneer in the field of sex hormones" to affidavit-like laudatory quotations from the pen of other prominent nutritionists. It would be expected that the biography of a friend would be most favorable from every point of view. Still, too much is made of the "implications" of some of Casimir Funk's work; it must not be forgotten that all scientific progress is based on building upon the work of others. A reader unfamiliar with the international picture of nutritional (and cancer) research in the past forty years might get the impression that Funk alone was the major force in these developments. It is obviously difficult if not impossible to assess the contributions of one scientist to current progress, especially if the scientist in question was a co-author in about a dozen papers.

The trials and tribulations of Casimir Funk nonetheless make interesting reading, for they show the difficulties that original ideas encounter when first presented, particularly if advocated by a person who is admittedly "opinionated" and who apparently worked better as a lone wolf. This friendly and sympathetic account of an individualistic scientist has within it some morals applicable to research today.

S.O.W.

Bread. The Chemistry and Nutrition of Flour and Bread with an Introduction to Their History and Technology, by Lord Horder, Sir Charles Dodds, and T. Moran. Constable, London, and the Macmillan Co., New York, 1954, pp. 186, $3.75.

During recent years the subject of bread has received much attention and much has been written about it. However, this is the first book which treats of this subject from the historical, theoretical, and practical standpoints. The book has additional merit, since the authors have themselves taken part in the many problems concerning bread which have arisen both during and since the last world war. The book deals with such diversified subjects as History of Bread, Wheats of the World, Chemistry of Wheat Flour and Bread, Main Features of the Milling and Baking Processes, Improving Agents, The Digestion and Assimilation of Bread, Bread and Nutrition, Enrichment of Flour and Bread, and Bread and Health.

The authors claim that the vitamin B_1 content of wheat, although apparently influenced by genetic factors, increases as the protein content rises, i.e., it increases about 0.3 μg/gram for each 1 per cent rise in protein. This observation (Greer, Ridyard, and Kent, *J. Sci. Food Agric.* 2: 12, 1952) needs confirmation.

Arginine and histidine are listed among the non-essential amino acids. Such classification needs an explanatory note that they are non-essential only for nitrogen equilibrium of the human adult. These amino acids may have other essential physiological functions for man.

A question often raised is: What influence does toasting have on the nutritional value of bread? The authors' answer is that toasting makes the amino acid, lysine, which is deficient in wheat, unavailable and has a marked destructive effect on the components of the vitamin B complex.

From the nature of its contents this book should be of interest to nutritionists, dietitians, bakers, and millers. BARNETT SURE

Annual Review of Medicine, Vol. 6, edited by D. A. Rytand and J. Anderson, Annual Reviews, Inc. Stanford, Calif., 1955, pp. 459, $7.00.

The Annual Review series has become established as a useful guide through the maze of the world's scientific literature. This volume, as its predecessors, maintains a high level of brief but wide coverage of the significant developments in many branches of medicine.

The chapter on Nutrition and Nutritional Disease was prepared by Lepkovsky and Borson. Some 218 references are documented, and among the topics are obesity, liver disease, pyridoxine, gestation, and the gastrointestinal tract. The subject and author indices are very useful, as is the annotated list of review articles.

This book continues to be unique in affording a critical bird's-eye view of the complicated panorama of modern medicine. S.O.W.

Vitamins and Hormones. Advances in Research and Applications. Vol. XII, edited by Robert S. Harris, G. F. Marrian, and Kenneth V. Thimann, Academic Press Inc., New York, 1954, pp. 305, $7.50.

This is another in what has become an outstanding series of survey articles. Most workers in the fields of nutrition, metabolism, and endocrinology are familiar with the high caliber of the reviews by recognized authorities which characterize this series. The present volume continues the tradition. Among the eight subjects covered are the chemistry of vitamin B_{12}, vitamin A requirements, hypervitaminosis A, and a contribution by a member of this editorial board, C. S. Davidson, on nutritional disturbances in liver disease.

In this reviewer's opinion, all serious students of endocrino-vitaminology should become familiar with these fine annual volumes, because they are among the best sources for the appreciation of recent advances in research and application. S.O.W.

Books received for review by the *American Journal of Clinical Nutrition* are acknowledged in this column. As far as practicable, those of special interest are selected, as space permits, for a more extensive review.

Feeding Your Child, by S. M. Wishik, Doubleday & Co., Inc., New York, 1955, pp. 223, $3.50.

Vitamins in Theory and Practice (4th ed.), by L. J. Harris, Cambridge University Press, 1955, pp. 336, $6.50.

Diseases of the Liver and Biliary System, by S. Sherlock, Charles C Thomas, Springfield, Ill., 1955, pp. 720, $10.00.

Nutrition for Practical Nurses, by P. S. Howe, W. B. Saunders Co., Philadelphia, 1955, pp. 174, $2.50.

Annotated Bibliography of Vitamin E (1952–1954), Vol. III, compiled by P. A. Harris and W. Kujewski, Eastman Kodak Co. (distributed by National Vitamin Foundation, Inc., New York), 1955, pp. 182, $3.00.

Nutrition and Diet Therapy (11th ed.), F. T. Proudfit and C. H. Robinson, The Macmillan Co., New York, 1955, pp. 854, $5.25.

Nutrition Sourcebook, compiled by O. E. Byrd, Stanford University Press, Stanford, Calif., 1955, pp. 370. $7.50.

Nutrition Practices: A Guide for Public Health Administrators, American Public Health Assoc., Inc., New York, 1955, pp. 80, $1.00.

The Body Fluids—Basic Physiology and Practical Therapeutics, by J. R. Elkinson and T. S. Danowski, Williams & Wilkins Co., Baltimore, Md., 1955, pp. 626, $10.00.

Allergy Cooking, by M. L. Conrad, Thomas Y. Crowell Co., New York, 1955, pp. 380, $5.00.

Abstracts of Current Literature

ABSTRACTERS

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ALCOHOL AND ALCOHOLISM

The utilization of alcohol as a source of calories apparently occurs at the two-carbon level (alcohol→ acetaldehyde), bypassing the glycolytic and fatty acid oxidation cycles. When alcohol is consumed in small quantity it appears to have no significant effect upon the oxidation of sugar or fats. For example, alcohol can be given in therapeutic amounts to well-controlled diabetics without disturbing their stabilization. In chronic alcoholism, however, liver function may be altered and hypoglycemia may ensue, probably due to the reduced content of glycogen in the liver.

Hypoglycaemia in Chronic Alcoholism. J. S. Taylor. *Brit. M. J.* 1: 648, 1954.

A case report is presented of a man of 54, known to be a chronic alcoholic, who was found in bed in deep coma. The only immediate abnormality appeared to be ketonuria. A tentative diagnosis of diabetic coma was made, but it was decided to give 25 ml of 25 per cent glucose intravenously before giving insulin. The man immediately regained conciousness. Later investigation showed a fasting blood sugar of 94 mg/100 ml, and the glucose tolerance test showed a maximum blood concentration of 171 mg/100 ml in 1½ hours, with a return to normal fasting values in 2½ hours. Liver function tests showed serum alkaline phosphatase 3 units, serum thymol turbidity 4 units, icterus index 3 units; the serum proteins were 9.8 mg/100 ml, the A/G ratio being 1.45:1.

It is suggested in discussion that both hypoglycemia and ketosis occurring together are consistent with diffuse hepatic damage, even though the function tests were not conspicuously abnormal.— F. E. HYTTEN

There is considerable question as to whether the calories derived from alcohol are capable of being utilized for energy formation. The rapid oxidation of alcohol within the body provides heat, which may be rapidly dissipated through vasodilatation. It would be of interest to know whether this will reduce the amount of calories derived from foodstuffs necessary for the formation of body heat, thus making more calories available for energy formation.

Is Alcohol an Energy Food? G. Laroche and J. Tremolières. *Bull. Acad. Méd.* 137: 369, 1953.

The authors show that alcohol does furnish calories, but these calories are not utilizable for muscular work except as exchangers in basal metabolism, and then only within variable and little-known limits.

Alcohol increases the consumption of oxygen by 10 to 12 per cent and produces a sensation of warmth, with peripheral vasodilatation.

The investigations of the National Institute of Hygiene lead to the conclusion that the alcohol supplied in the following amounts of a 10° wine to heavy laborers—0.94, workmen—0.74, employees—0.59 ml is probably not dangerous and seems to be reasonable.— H. GOUNELLE

The increased blood flow secondary to vasodilatation occurring after alcohol ingestion may be observed in the splanchnic circulation as well as in the periphery.

Effect of Intravenous Infusions of Ethanol upon Estimated Hepatic Blood Flow in Man. A. I. Mendeloff. *J. Clin. Investigation.* 33: 1298, 1954.

An intravenous infusion of 5 per cent alcohol in saline was administered to 5 subjects with peptic

ulcer. The hepatic blood flow was measured by the hepatic vein catheterization and bromsulfalein (BSP) technique. Under the conditions of this study, there was a significant increase in hepatic blood flow over values obtained after saline alone. These findings can best be explained as due to a lowering of the peripheral resistance of the splanchnic vascular bed.

Other evidence obtained showed no subjective effects from the rather small doses used. There was minimal peripheral disappearance of alcohol, most subjects showing significant hepatic uptake. The findings are also in agreement with current concepts of the conversion of alcohol to acetaldehyde.—S. O. WAIFE

Sodium retention in alcoholic cirrhosis with ascites has been attributed in the past to the action of certain adrenal steroids. Recently aldosterone has been shown to be elevated in the urine of patients with this condition. The increased level of this steroid, however, may be a secondary effect of the disturbed fluid and electrolyte metabolism in these subjects.

Study of the Renal Elimination of Sodium in Alcoholic Cirrhoses with Ascites. R. Cachera, F. Darnie, and J. Lubetzki. *Sem. Hôp. Paris* 70: 3613, 1953.

The urinary elimination of sodium, always slight in alcoholic cirrhosis with ascites, shows a certain parallelism with the extent of the hepatic disorder. The quantity of sodium filtered per minute has been found to be markedly decreased. It becomes minute in cases in which ascites develops rapidly. In the cirrhotic, furthermore, the proportion of filtered sodium reabsorbed is increased. The independence between the reabsorption of water and that of sodium is particularly clear during the course of osmotic diuresis. In ascitic cirrhoses, following overloading with sodium chloride, the quantity of sodium reabsorbed is elevated with a concomitant rise in natremia, while it remains relatively stable under these conditions in the normal subject.

Inability to increase the spread between the level of plasma sodium and the quantity reabsorbed per 100 ml of filtrate seems rather peculiarly typical of cirrhotics. This leads to the stressing of hyponatremia among the factors determining the degree of sodium reabsorption.—H. GOUNELLE

The histopathological changes in the liver induced by alcohol are frequently more impressive than the alterations seen in hepatic function until late in the course of the process. The question as to whether alcohol induces these changes directly as a toxic substance or as a source of "empty" calories causing secondary nutritional changes is still actively debated.

Alcoholic Steatosis. Relations Between Steatosis and Cirrhosis. R. Cachera, M. Lamotte, and F. Darmis. *Ann. Nutrition & Alimentation* VII (5): C 130, 1953.

Over-deposition of lipids in the liver, attaining the degree of a massive fatty infiltration, is the dominant anatomical characteristic of simple alcoholic hepatitis without any clinical sign of cirrhosis. The good tolerance of these cases of fatty infiltration, even when massive, is the most striking clinical finding. Various tests have demonstrated the habitual absence of marked disturbances of the hepatic functions investigated. One fundamental fact can be stated: a cirrhotic ascites can exist when the histologic changes are limited to a simple reticular hyperplasia or even to a marked fatty infiltration with minimal fibrosis.—H. GOUNELLE

Histobiologic Syndromes in Chronic Alcoholic Hepatitis. G. Albot, R. Dupuy, J. Herman, and M. Corteville. *Sem. Hôp. Paris* 40: 2533, 1954.

Pre-cirrhotic alcoholic hepatitides may be described from a double standpoint—anatomic and biologic—in terms of three distinct histobiologic syndromes:

(1) *Isolated anatomic fatty infiltration.* The histologic appearance is that of a fatty infiltration of the parenchyma without any important modification of the mesenchyme. The state of hepatic function is variable; the most frequently encountered changes are those of diuresis, galactosuria, and hyperproteinemia—with increase in the globulin fraction.

(2) *Cirrhogenous hepatitis with fatty infiltration.* The histologic picture associates fatty infiltration and architectural destruction of the lobular arrangement with the development of nodules of hyperplasia. Biologically, the disturbances affect, at the same time, diuresis, galactosuria, and urinary hyppuric acid. The Kunkel test is always normal, the R.C.T. is always strongly positive, indicating a mesenchyme reaction.

(3) *Cytolytic cirrhogenous hepatitis.* Histologically, the appearance is comparable to the preceding, except for the fact that there is no fatty infiltration, or very little. Biologically, results of all liver function tests are abnormal, with one special distinction consisting in the very marked positivity of all flocculation tests, including the Kunkel test.—H. GOUNELLE

Continuing studies dealing with possible genetic factors in alcoholism are important to those interested in the pathogenesis of alcoholism.

Dietary Deficiencies in Animals in Relation to Voluntary Alcohol and Sugar Consumption. R. J. Williams, R. B. Pelton, and L. L. Rogers. *Quart. J. Studies on Alcohol* 16: 234, 1955.

Dietary deficiencies have been shown to increase the voluntary consumption of alcohol by rats, but the same deficiencies do not have the same effects in all rats. There seem to be genetic determinants of the individual rat's response to specific vitamin deficiencies. These constitutional differences are probably operative in human beings as well as in experimental animals, and we must allow for individual factors in our approach to the complex problem of alcoholism.

Continuing their investigations of the effect of diet on alcohol consumption by rats, the authors fed two groups of Wistar rats (15 rats in each group) a basal diet supplemented so as to be either rich or poor in vitamins. The experiment was divided into three periods of about 28 days each. During the first period, the rats were given their choice of 10 per cent sucrose solution or distilled water, *ad libitum;* during the second and third periods, a 10 per cent alcohol solution replaced the sucrose solution. Group I was started on the vitamin-poor diet, Group II on the vitamin-rich one; the diets were switched for the third period. As we have come to expect in such experiments, certain rats showed a high degree of individuality: one rat, for instance, drank only 4.86 ml/day of sugar solution on the vitamin-poor diet, while a fellow-rat consumed 47.17 ml/day on the same regimen. And although the Group I rats in general drank much less alcohol in the third experimental period, when they were switched to the vitamin-rich diet, two obstinately alcoholic rodents drank approximately 18 times as much alcohol as the most abstinent members of the group. Unless one assumes that some rats are highly "neurotic," it is hard to explain these different patterns of consumption except on an individual genetic basis, as Williams does.

Despite the erratic behavior of individual rats, several conclusions can be drawn from the experiment. One is that sugar consumption, as well as alcohol consumption, is increased by a deficient diet, and that excessive sugar consumption aggravates the deficiency and results in diminished growth. It was also observed that the Group II rats, who were started on the vitamin-rich diet, did not increase their consumption of alcohol to any appreciable extent when they were switched to the deficient diet—indicating that a previous good diet prevents the rapid development of deficiency in mature animals. Other workers have shown that rats differ from humans in that if placed on a deficient diet and given their choice of sugar solution or alcohol, they will choose the sugar solution. In this experiment it became apparent that rats with a "sweet tooth" are not necessarily the ones who become large consumers of alcohol when deprived of sugar. Indeed, the rat who consumed the least sugar in the first period of this experiment consumed the most alcohol in the last.

It is postulated that differences in sugar metabolism in rats and humans may account for some of the observed differences in their beverage choices during deficiency, but the authors hold that the same general principle operates in both animals and men; that is, inadequate nutrition impairs the "food selection mechanisms," perhaps by some interference with hypothalamic control, and adequate nutrition leads to "wiser" choices.—C.-J. HOWELL

The use of chlorpromazine holds promise in the management of acute alcoholism, facilitating an early retention of food and nutritional supplements.

Chlorpromazine in the Treatment of Acute Alcoholism. E. H. Mitchell. *Am. J. Med. Sc.* 229: 363, 1955.

This study was made on 306 patients representing 400 admissions for treatment of acute alcoholism. One half of the cases received chlorpromazine as the primary therapeutic agent and one half were sedated with barbiturates and mephenasin. The former group was given 50 mg intramuscularly or by vein, following which they received 50 to 100 mg orally every four hours. The latter group received 250 mg of secobarbital intravenously and butabarbital and 100 mg of mephenasin every 3 hours until bedtime. In those receiving chlorpromazine, mild postural hypotension developed in 43 instances and severe hypotension in 7 instances. Lowering of the dose of the drug controlled the hypotensive effects. Tachycardia, noted in 22 cases, disappeared when the drug was stopped. There was one death in the chlorpromazine group in a poor risk cardiac patient with hepatic cirrhosis. The results obtained in the chlorpromazine group were encouraging in 85 per cent of the 200 cases studied. There was an effective sedative effect; nursing care was simplified and patients were more amenable to treatment; food was retained earlier, and discharge from the hospital was effected on an average of 24 hours earlier than in the barbital group.—C. R. SHUMAN

Chlorpromazine in the Management of Acute Alcoholic States. J. D. Schultz, E. L. Rea, J. F. Fazekas, and J. C. Shea. *Quart. J. Studies on Alcohol* 16: 245, 1955.

Chlorpromazine has definitely proved its usefulness in the management of acute alcoholic states. Administered to 164 patients admitted to the alcoholic service of the District of Columbia General Hospital, it not only controlled motor excitement but permitted the early institution of nutritional and other appropriate measures. Since adequate nutrition is of the utmost importance in treating acute alcoholism, chlorpromazine's ability to control vomiting and nausea makes it an invaluable therapeutic adjunct. Even patients who had been vomiting almost continuously were rendered capable of retaining food and fluids they would otherwise have been unable to tolerate, and usually they were eating well inside of 24 hours. The standard dose in this series was 100 mg intramuscularly on admission and 50 mg three times daily until symptoms disappeared. Intra-

nous injections proved more effective in severely disturbed patients. In some cases the standard dose had to be increased to 75–100 mg/day, and the total dosages ranged from 100–2950 mg over a period of one to six days. On these doses there were no severe or alarming side-effects, except for three serious hypotensive reactions. These, as the authors point out, can be avoided by keeping the patient at bed rest and by checking the blood pressure frequently.—C.-J. HOWELL

REGULATION OF CHOLESTEROL LEVELS

The concentration of cholesterol in the blood depends upon the rate of production and absorption versus the rate of its removal from the plasma. The rate of production of endogenous cholesterol appears to be dependent upon the amount required to maintain a homeostatic level. Thus production increases as exogenous supplies are decreased. However, absorption is dependent upon the level of dietary fat, so that dietary cholesterol or that introduced into the intestine from the liver by biliary excretion requires fat, primarily of animal origin, to be absorbed. An upward trend of blood cholesterol is linked with a rising fat/calorie ratio in the diet. Evidence has been presented to show that isocaloric substitution of plant fat for animal fat results in a marked lowering of cholesterol levels even when this substance is added directly to the diet. The removal of cholesterol involves fecal excretion, uptake by reticuloendothelial cells, and utilization as in steroidogenesis.

Pathogenesis of Dietary-Induced Hypercholesteremia in the Rabbit. S. O. Byers and M. Friedman. *Am. J. Physiol.* 179: 201, 1954.

Rabbits and rats were compared by carrying out identical studies on each species. The rabbit is susceptible to dietary hypercholesteremia, while the rat is resistant to it. These studies demonstrate that rabbits absorb a larger fraction of ingested cholesterol than does the rat. Even though the ingested absorption fraction in the rat was increased to a value close to that in the rabbit, the former animal did not have a hypercholesteremia during the first three days. Furthermore, when rabbits were maintained on oral cholesterol equivalent to the fraction absorbed in rats, the former animals were observed to have an elevated blood cholesterol. Thus, increased absorption of cholesterol from the intestine does not have the most important role in the rabbit, which has a hypercholesteremia following orally administered cholesterol. It should be pointed out, however, that when rats are forced to ingest large amounts of cholesterol they do have an observed moderate hypercholesteremia after a long delay.

The rabbit is unable to remove cholesterol easily from its plasma. It does not matter whether this material is derived from the intestine or injected intravenously. This is the most important cause of hypercholesteremia in this species. Cholesterol remains in the rabbit's blood and does not enter the liver, as is the case in the rat. The parenchymal cells of the liver of the rabbit do not seem to be the locus of the difficulty. Serum of rabbits containing large amounts of cholesterol was administered to rats. These animals removed the cholesterol as well as they did from rat plasma. There is no abnormality in rabbit's plasma itself. Rat's serum was unable to block dietary hypercholesteremia in rabbits. When rat serum was largely replaced by that of a rabbit it did not result in a greater degree of hypercholesteremia when cholesterol was ingested.

Rabbit and rat plasma were equally effective in clearing turbid sera, and each was cleared with equal ease. Heparin was ineffective in both species in relation to removing injected cholesterol, nor did it prevent hypercholesteremia following ingestion of cholesterol in the rabbit.

The authors point to recent work which implicates the hepatic reticuloendothelial system in the deposition of dietary cholesterol. This last should be differentiated from the way endogenous cholesterol is handled. This difference depends on the fact that dietary cholesterol is carried initially as an insoluble moiety of lipid chylomicra in the blood stream. The same is not true of endogenous cholesterol. Chylomicra are of large size and insoluble. As a result, they must be reduced in size and made soluble in order to enter polygonal cells of the liver. To do this, chylomicra must be "cleared" or ingested by Kupffer cells to break them down. Rabbit reticuloendothelial system is deficient. This may involve the number of active cells or may be a functional matter. In the rabbit, the reticuloendothelial system does not ingest chylomicra rapidly. Therefore, chylomicra are not removed from plasma to enter parenchymal liver cells. As a result, hypercholesteremia is persistent in this species after cholesterol feeding. Hyperlipemia and turbidity can be similarly explained. Lipids are carried in chylomicra and these aggregations themselves are responsible. This concept is supported by the fact that when one interferes with the rat's reticuloendothelial system this animal behaves like the rabbit in the face of increased ingestion of cholesterol.—M. J. OPPENHEIMER

Fatty Acid and Cholesterol Synthesis Rates in the Intact Rat. T. T. Hutchins, J. T. Van Bruggen, and E. S. West. *Arch. Biochem. and Biophys.* 52: 261, 1954.

This report introduces an approach to the determination of lipid synthesis rates in the individual intact rat using single tracer injection, short term experiments. In addition to the determination of the fraction of administered C^{14} incorporated into fatty acid and cholesterol, use is made of respiratory $C^{14}O_2$ data to evaluate the carbon flux through precursor

stages. Fatty acid and cholesterol synthesis rates of rats in postprandial, postabsorptive, and fasted states are reported. Methods for the calculation of lipid and cholesterol half-times are presented. The assumption that a C_2 acetylating agent—derived from pyruvate, fatty acids, certain amino acids, and exogenous acetate—serves as the carbon source for all carbon atoms for fatty acid and cholesterol synthesis is discussed.—M. K. HORWITT

Role of Fat and Fatty Acid in Absorption of Dietary Cholesterol. L. Swell, D. F. Flick, H. Field, Jr., and C. R. Treadwell. *Am. J. Physiol.* 180: 124, 1955.

Soybean oil was hydrogenated to varying degrees and fed to rats in diets which did not contain cholesterol. Blood cholesterol varied with the iodine number; the higher the unsaturation, the higher was the blood cholesterol level. Blood cholesterol was elevated more by free fatty acids than by neutral fats. Cholesterol is esterified more rapidly with fatty acids than with neutral fats in the pancreatic cholesterol esterase system. Dietary fat is not needed to ensure cholesterol absorption if bile salts are present. Endogenous sources may furnish fatty acids utilized for esterification. The authors suggest that dietary fat stimulates a flow of bile and that this is used for cholesterol esterase activity. At the same time, dietary fat provides fatty acid for esterification of cholesterol.—M. J. OPPENHEIMER

Absorption of Dietary Cholesterol Esters. L. Swell, T. A. Boiter, H. Field, Jr., and C. R. Treadwell. *Am. J. Physiol.* 180: 129, 1955.

Observations were made on blood cholesterol. A series of rats which were fed cholesterol and cholesterol butyrate were observed to have a greater increase in cholesterol than a similar group which was fed cholesterol oleate and linoleate. Diets which included sodium taurocholate produced more marked elevations in blood cholesterol. Cholesterol butyrate is rapidly split. On the other hand, the oleate and linoleate are split more slowly. This leads the authors to suggest that cholesterol must be in the free form for absorption.—M. J. OPPENHEIMER

Physicochemical differences in the form of cholesterol arising from endogenous or exogenous sources may be of importance in the rates of deposition in blood vessels. One would assume that the less soluble exogenous cholesterol may be more important as a cause of atherosclerosis.

Observations Concerning the Production and Excretion of Cholesterol in Mammals. XIII. Role of Chylomicra in Transport of Cholesterol and Lipid. S. O. Byers and M. Friedman. *Am. J. Physiol.* 179: 79, 1954.

Dietary absorbed cholesterol was observed to travel in intestinal lymph almost entirely as chylomicrons, that is, in particulate form. Much dietary absorbed cholesterol is in particulate form in plasma in animals which have been fed cholesterol. When cholesterol is produced endogenously it is in a soluble form in plasma. This is true in both normal controls and hypercholatemic conditions. Part of the cholesterol synthesized in nephrotic rats is in particulate form in plasma.—M. J. OPPENHEIMER

In hyperthyroidism, the turnover of cholesterol and other lipids is increased. The enhanced rate of disposal of cholesterol accounts for the reduced serum levels in this disease.

Influence of Thyroid Activity on the Hepatic Excretion of Cholic Acid and Cholesterol. J. C. Thompson and H. M. Vars. *Am. J. Physiol.* 179: 405, 1954.

The amount of bile obtained from a cannula in the bile duct was related to food intake. Hyperthyroid rats excreted more cholesterol in the bile than did control animals. Hypothyroid animals secreted less. Cholic acid biliary secretion was decreased in hyperthyroidism but unchanged in depressed thyroid function. In two pair-fed series (thyroidectomized; propylthiouracil-treated), cholic acid was markedly increased.—M. J. OPPENHEIMER

In humans, caloric restriction generally means a low fat and low cholesterol intake, which has been found associated with low serum cholesterol levels and a reduced incidence of arteriosclerosis. The experiment reported here suggested that caloric restriction does not protect if cholesterol intake is elevated.

Effect of Caloric Restriction on Cholesterol Atherogenesis in the Rabbit. M. G. Goldner, L. Loewe, R. Lasser, I. Stern. *Proc. Soc. Exper. Biol. & Med.* 87: 105, 1954.

In spite of severe caloric restriction cholesterol feeding in rabbits resulted in increased deposition of lipids in the intima, and increased levels of blood cholesterol, fatty acids, lipoproteins, and phospholipids. It would appear that in this species weight loss *per se* does not protect against the effect of high cholesterol feeding, and, as a matter of fact, seemed to enhance cholesterol-induced atherosclerosis.—L. W. KINSELL

The Effect of Essential Fatty Acid Deficiency on the Distribution of Endogenous Cholesterol in the Plasma and Liver of the Rat. R. B. Alfin-Slater, L. Aftergood, A. F. Wells, and H. J. Deuel, Jr. *Arch. Biochem. and Biophys.* 52: 180, 1954.

Rats were depleted of essential fatty acids by being placed on low fat diets which were nutritionally complete except for the deficiency in unsaturated fatty acids. The control rats were fed a similar diet containing 12.5 per cent of refined cottonseed oil. After

20 weeks, a significant increase was found in the cholesterol content of the livers from rats receiving the low fat diet, as contrasted to those from rats receiving the fat-supplemented ration. The cholesterol content of the plasma decreased. A similar, but less marked increase was observed in the adrenals. The increase in the cholesterol of the livers of fat-deficient rats was predominantly in the ester fraction, although some increase in free cholesterol was found.

It was suggested that in the absence of unsaturated fatty acids the increase in the esterified cholesterol content of the livers was due to the formation of esters with other more saturated fatty acids and that these esters may not be available for metabolism in the liver; therefore, they accumulate in this organ.—M. K. HORWITT

Because of difficulties encountered in enforcing low animal fat dietary programs, continued interest is displayed by clinicians and investigators in substances which will interfere with cholesterol absorption during normal feeding. Such substances may include phenyl-ethyl-acetic acid, mineral oil, the unsaturated fatty acids, and other compounds.

Therapeutic Trial of a Synthetic Cholesterolemia-Lowering Substance: Phenyl-Ethyl-Acetic Acid. J. Cottet, A. Mattivat, and J. Redel. *Presse méd.* 62: 939, 1954.

The authors, after consideration of the exogenous and endogenous origin of cholesterol and its role in atherosclerosis, are in agreement with the opinion of the majority of authors that this substance plays a not exclusive but certainly important part in the development of atherosclerosis.

They show that phenyl-ethyl-acetic acid lowers cholesterolemia, both experimentally and clinically in a study of 101 cases.—H. GOUNELLE

Effect of Mineral Oil on Intestinal Absorption of Cholesterol in the Rat. E. Karvinen, T. M. Lin, and A. C. Ivy. *Am. J. Physiol.* 181: 439, 1955.

Light mineral oil was added at a level of 6 per cent of the dry weight of the diet. Under this experimental condition, fecal excretion of endogenous cholesterol was 39 per cent greater than when mineral oil was not added. If cholesterol was added to the diet, then mineral oil increased the cholesterol excretion 30 per cent. Since cholesterol is soluble in mineral oil, up to 1.1 per cent at 37° C., this may explain the increased excretion. Laxation is probably another factor which operates to increase excretion.—M. J. OPPENHEIMER

Effect of Essential Unsaturated Fatty Acids and Methionine on Hypercholesteremia. S. L. Shapiro and L. Freedman. *Am. J. Physiol.* 181: 441, 1955.

When rats on a sulfur-deficient protein diet are fed cholesterol, a hypercholesteremia is observed. This may be reduced or prevented if safflower oil, which is high in essential unsaturated fatty acids, is administered along with a methionine supplement. Under similar experimental conditions, a hydrogenated fat was ineffective. It is pointed out that diets rich in unsaturated fatty acids cause a marked fall in serum cholesterol levels. The low incidence in Chinese of atherosclerosis and their relative hypocholesterolemia has been ascribed to their high intake of unsaturated fatty acids. The authors express the opinion that the "incorporation of 2.5% by weight of the total diet, of vegetable oil high in content of sterically required isomers of essential unsaturated fatty acids is a factor of importance in the design of diets intended for treatment and prevention of atherosclerosis. The use of fats containing these acids beyond caloric levels where they comprise 10–15% by weight of the diet, undoubtedly affords the undesirable caloric pattern described by Keys."—M. J. OPPENHEIMER

GERIATRIC NUTRITION

The bulk of the abstracts published in this section derive from an excellent survey of the nutritional status of an elderly population group residing in a progressive California community. In general, there is a close correlation between dietary intake of the nutritional factors analyzed and the biochemical data obtained. At optimal levels of food intake, essentially normal values are found for hemoglobin, vitamins, and protein. The direct correlation described between dietary fat and serum cholesterol is an anticipated finding, as is the lack of correlation between blood glucose and dietary intake. A gradual reduction in renal function with increasing age has been reported previously, which may account for the increased blood creatinine and nonprotein nitrogen over that found in young adults.

Nutritional Status of the Aging. I. Hemoglobin Levels, Packed Cell Volumes and Sedimentation Rates of 577 Normal Men and Women Over 50 Years of Age. H. L. Gillum and A. F. Morgan. *J. Nutrition* 55: 265, 1955.

This study was part of the western regional cooperative project on nutritional status of selected population groups. The California phase of the research was carried out in 1948–49 on 280 men and 297 women over 50 years of age, residing in San Mateo County, California. This area was chosen because of its well organized department of public health and welfare, because its people were thought to be relatively stable as to residence, and because of its proximity to the home laboratory. The object of the study was to determine the food habits of aging people, to learn whether these habits could be connected in any way with their physical condition, blood composition, density of bone, and other measurable characteristics. The number of subjects re-

ported upon for various aspects of the study will vary somewhat, since all determinations were not made or were not accepted for each of the 577, the maximum number examined.

Physical examinations, medical and dietary histories, 7-day diet records and blood and urine analyses were carried out on these men and women. More than 80 per cent were in comfortable middle-class circumstances, and all but 47 men were living in their own homes. These 47 men lived in the county home.

The hemoglobin levels of the men living in their own homes were higher than those of the women, 14.5 ± 0.3 g per 100 ml of blood as compared with 13.4 ± 0.3. In the groups 75 years of age and older, however, the values declined in both men and women and the difference between them became insignificant. The mean values were 1 to 2 g per 100 ml lower than those usually reported for young adults, but the discrepancy was greater in men than in women.

The volume of packed cells was likewise larger in men than in women, 47.1 ± 0.7 per cent in men living in their own homes and 44.6 ± 0.5 in women. The mean corpuscular hemoglobin concentration for these men and women was 31 and 30 per cent, somewhat lower than in young adults, and due to the apparently increased cell size in the older subjects.

The sedimentation rates were higher in women than in men and tended to increase with advancing age. Eighty-four per cent of the men and 52 per cent of the women had rates 0 to 20 mm per hour, and 16 per cent of the men and 48 per cent of the women 21 to 50.

The diet records indicated that the men living in their own homes ate from 15 to 34 per cent (mean 27) more calories and protein and 0 to 50 per cent (mean 26) more iron per kilogram body weight than the women in the same age groups. The protein calories were 14 per cent of the total in both sexes and the iron intake 6.0 and 6.1 mg per 1000 calories. The qualitative nature of the diets was apparently the same. A trend toward decrease with age in intake of these nutrients was evident.

The majority of the higher intakes of both protein and iron were found in the groups with higher levels of hemoglobin, and the lowest intakes in those with the lowest hemoglobin values. The positive correlation between dietary protein and hemoglobin was +0.12, and between iron and hemoglobin +0.13, both significant at the 1 per cent level.—B. Sure

Nutritional Status of the Aging. II. Blood Glucose Levels. H. L. Gillum, A. F. Morgan, and R. I. Williams. *J. Nutrition* 55: 289, 1955.

Of 577 presumably normal men and women over 50 years of age examined as to dietary intake, physical condition, and blood and urine composition, postprandial blood sugar levels of 430 subjects are reported. All records used in computation of means were of persons who had eaten a carbohydrate meal two hours or more previous to withdrawal of the venous blood sample. Forty-five men were living in the county home and the rest of the subjects in their own homes. The mean venous blood sugar levels, excluding all over 130 mg per 100 ml, determined by a method which recorded the true reducing sugar content, calculated for 5-year age intervals and separated as to sex showed little variation with age or sex, although there was a slight indication of an increase with age in the women. The overall mean was 101 mg per 100 ml of blood. This is significantly higher than the means usually quoted for young adults. Hyperglycemia judged by various criteria was found in 2.1 to 5.6 per cent of men and 1.3 to 4.4 per cent of the women. Glycosuria of varying degrees was noted in 36 cases, 24 of them two hours or more after the last meal. In 18 of these cases the blood sugar level was over 130 mg per 100 ml of blood, and in 18 it was under that amount. Moderate to severe glycosuria and hyperglycemia occurred together in 8 men and 2 women. Four women and 14 men had equally severe glycosuria but normal or low blood sugar levels. Only 5 of these persons were known previously to be diabetic. Variable renal reabsorption of glucose may account for the discrepancies in blood and urine findings.

The effect on distribution of the blood sugar levels of food intake at one-half hour intervals previous to the withdrawal of the blood sample was noted in a group of 167 persons.

The men in the county home over 65 years of age had significantly lower blood sugar levels (mean 85 mg per 100 ml of blood) than men of the same age living in their own homes. There were 6 hyperglycemic and 3 diabetic cases also in the county home group. This group presented an unusual incidence of diabetes and its presence in the sample may account for the apparent higher incidence of these signs in men than in women.

A moderate positive correlation between fat intake and blood sugar level was found in the women but not in the men. No correlation of blood sugar with carbohydrate or protein intake was found in either men or women.—B. Sure

Nutritional Status of the Aging. III. Serum Ascorbic Acid and Intake. A. F. Morgan, H. L. Gillum, and R. I. Williams. *J. Nutrition* 55: 431, 1955.

Many intake records and estimates as well as experimental studies of blood levels and urinary excretion of ascorbic acid under controlled conditions have appeared during the last 20 years. However, questions which have still to be answered concern the magnitude of the optimum intake and plasma levels, the true significance to health of saturation of the tissues with ascorbic acid, the metabolic function of this vitamin, and the effect of sex, age, chronic disease, drugs, and other stresses on its utilization.

The serum ascorbic acid levels, 7-day records of ascorbic acid intake, and physical condition, particu-

larly of teeth and gums, were obtained for 569 supposedly healthy men and women over 50 years of age. All of these were living in their own homes except for 44 men over 60 years old living in the county home. The serum levels and daily intake values were grouped for men and women separately in 5-year age groups from 50 to more than 80 years of age. The women at all ages had higher serum ascorbic acid levels than the men, means 1.07 ± 0.08 and 0.83 ± 0.08 mg per 100 ml, respectively. In both sexes a maximum was noted in the age group 60 to 64 years. The total daily intakes of the men, however, were greater at all ages, means 99 ± 0.10 mg for the men and 86 ± 0.08 for the women. The daily intakes per kilogram of body weight were 1.36 ± 0.14 and 1.28 ± 0.12 mg. Expressed thus, the difference in intakes was not significant. The 44 men in the county home had only 0.27 ± 0.06 mg per 100 ml serum ascorbic acid and 40 mg total daily intake or 0.62 mg per kilogram of body weight.

A direct correlation, $r = 0.46$, was found between the serum levels and intakes of the whole group. Seventy to 75 per cent of all men and women having less than 0.5 to 0.7 mg per 100 ml of serum ascorbic acid, respectively, were in the lowest intake group with less than 69 mg of ascorbic acid in their daily food. Conversely, 75 per cent of the men and 50 per cent of the women with intakes of this lowest level had serum ascorbic acid levels also in the lowest brackets.

An approximation of economic level was recorded with 44 men in the county home in one group, 146 persons on relief and old age assistance rolls in a second group, the middle income group of 396 persons in another, and those in the highest income group, 23 persons, in a fourth group. The mean serum ascorbic acid levels of these four groups progressed from low to high in this same order. Ninety-one per cent of the men in the county home, 40 per cent of those on relief and old age pensions, 21 per cent of those with the middle incomes, and none of those with the high incomes had serum levels less than 0.5 mg per 100 ml.

Vitamin supplements which might have contained ascorbic acid were taken more or less regularly by 18 per cent of the men and 11 per cent of the women, but these additions were not included in the intake calculations.

Thirty-nine per cent of the people examined were edentulous, 17 per cent had gingivitis in some degree, and 43 per cent had teeth and no gum disease. There was a consistently greater proportion, 24 per cent of the edentulous and of those with gingivitis, in the group with serum ascorbic acid below 0.3 mg per cent than of those with healthy mouths, 9 per cent. A higher proportion of those with teeth and healthy gums, 54 per cent, was found in the group with the higher serum levels, over 0.7 mg per 100 ml. Thirty-four per cent were edentulous and 12 per cent had gingivitis in this group.

The ascorbic acid requirement of men beyond 50 years of age appears to be significantly greater than that of women.—B. SURE

Nutritional Status of the Aging. IV. Serum Cholesterol and Diet. H. L. Gillum, A. F. Morgan, and D. W. Jerome. *J. Nutrition* 55: 449, 1955.

Much attention has been focused in recent years on serum cholesterol levels, particularly in aging persons, because of their possible relationship to the development of atherosclerosis. Correlations between serum cholesterol levels and physical measurements, such as body weight and blood pressure, and dietary intakes, particularly of fat, cholesterol, and protein, were sought in this study.

The serum cholesterol levels of 530 supposedly normal persons, 234 men and 296 women, 50 to more than 80 years of age, living in their own homes, were found to vary from 106 to 720 mg per 100 ml blood, the means for the men being 241 ± 8 and for the women, 270 ± 8. The free cholesterol was 27 to 28 per cent of the total and this proportion did not vary significantly in any group. The mean level for 43 additional men over 60 years of age, living in the county home, was 209 ± 12.

The women had significantly higher levels than the men between the ages of 60 and 80 years. In both sexes a sharp drop in the levels occurred at 75 or 80 years. There was a downward trend in the levels of the men with each decade, but a sustained high level was shown by the women in the groups from 60 to 75 years of age, after a sharp drop in the 54- to 59-year group. This drop may be due to a post-menopausal spurt in steroid hormone production.

A positive correlation of the order of 0.12 was found between dietary cholesterol and serum cholesterol levels in both men and women and a similar correlation, 0.15 in men, 0.09 in women, between fat intake and serum cholesterol levels. When intake of fat of animal origin only was considered, the correlation was lower. A slight positive correlation with protein intake was also found, and this was thought to stem from the similar dietary occurrence of fat, cholesterol, and protein.

A striking parallelism was noted between the serum ascorbic acid and serum cholesterol levels in women but not men. A possible relationship between these blood constituents and steroid hormone manufacture and circulation is suggested.

Extreme under- or overweight, 20 per cent or more, in men but not in women, was found to be associated with low and high serum cholesterol levels, respectively, as well as with low and high intakes of fat and cholesterol. No such relationship was seen in the groups with smaller deviations from the normal weight.

No relationship between blood pressure and serum cholesterol levels was found in any of the groups. It is clear that standards for hypercholesteremia must be different for men and women and that dietary fat

and cholesterol are associated positively with the serum cholesterol levels.—B. SURE

Nutritional Status of the Aging. V. Vitamin A and Carotene. H. L. Gillum, A. F. Morgan, and F. Sailer. *J. Nutrition* 55: 655, 1955.

Serum vitamin A and carotene determinations, physical examinations, and 7-day dietary records were obtained on 514 supposedly healthy men and women over 50 years of age. Thirty of the men were living in the county home, the others in their own homes. A small decline in both vitamin A and carotene serum levels occurred with age in both men and women, the mean values by decades being, in micrograms per 100 ml, 60, 54, and 50 for the men, and 57, 57, and 50 for the women. The corresponding carotene values were 119, 116, 109 for the men, and 123, 127, 113 for the women. No significant sex differences were found. Only 5.6 per cent of the subjects had serum vitamin A levels below 30 μg per 100 ml and 5.2 per cent above 80. Nearly half of both men and women had serum vitamin A levels between 40 and 60 μg per 100 ml. The serum carotene levels varied more widely, with about 62 per cent falling between 60 and 150 μg per 100 ml.

The dietary intakes were calculated for preformed vitamin A and for carotene separately and the probable vitamin A equivalent determined. This was found to be 2.4 μg per day for the men living in their own homes, 1.8 for the women, and 1.4 for the men in the county home. This amounted to 33, 27, and 21 μg per kilogram of body weight. Twenty-seven per cent of the intake of the men living in their own homes was from carotene, 29 per cent of that of the women, and 18 per cent of that of the men in the county home. About 11 per cent of the men and 20 per cent of the women took vitamin A supplements more or less regularly. When these were included, the average total intakes were 11,470 and 10,200 IU daily for the men and women living in their own homes as compared with 10,640 and 8450 when food sources only were used.

A slight positive correlation between total vitamin A intake and serum vitamin A level was established for both men and women. The correlation between serum carotene and carotene intake was more pronounced, $r^2 = 0.37$. Thickening of the bulbar conjunctiva was noted in 94.1 per cent of the subjects, but this was not more marked in those with low than in those with high serum levels of vitamin A. The same was true of the other changes in the conjunctiva and skin. There was more relationship to age than to vitamin A levels or intakes in the distribution of these signs.—B. SURE

Nutritional Status of the Aging. VI. Serum Protein, Blood Non-Protein Nitrogen, Uric Acid and Creatinine. A. F. Morgan, M. Murai, and H. L. Gillum. *J. Nutrition* 55: 671, 1955.

The total serum protein levels were determined in 573 men and women over 50 years of age and the nonprotein nitrogen, uric acid, and creatinine levels in the blood of 255 of these. Seven-day diet records and physical examinations were also recorded. The average serum protein level was 6.47 $\pm$ 0.06 g per cent in the men and 6.44 $\pm$ 0.05 in the women. The range was 5.2 to 7.4, but about 90 per cent of all values fell between 6.0 and 7.0. These levels are 10 to 15 per cent lower than those usually quoted for young adults. No correlation with protein intake was found, nor was there any age or sex difference, but there was some positive relationship to hemoglobin levels.

The blood nonprotein nitrogen was greater in the men than in the women, 38 $\pm$ 1.4, and 34 $\pm$ 1.2, but after 70 years this difference was not significant. In the men but not in the women the nonprotein nitrogen levels were positively correlated with protein intake. The mean levels were about 40 per cent higher than those usually found in young adults. The blood uric acid levels were also higher in men than women but after 70 years the differences were less marked and the levels decreased. The mean values 3.24 $\pm$ 0.19 mg per cent in men and 2.98 $\pm$ 0.18 in women were 28 per cent larger than those found by the same method in a group of young men and women, 2.53 and 2.33 mg per cent. There was no correlation with the fat intake but a small positive correlation with protein intake in the men but not in the women.

The blood creatinine levels were not affected by sex or age. The mean values were 1.53 $\pm$ 0.11 and 1.50 $\pm$ 0.08 mg per cent for men and women, respectively. No correlation with protein intake could be detected. These levels are 20 to 30 per cent higher than those usually quoted for young adults or for the group of 23 young people examined in this study, 1.27 $\pm$ 0.10 and 1.18 $\pm$ 0.07 mg per cent for young men and women, respectively. The data suggest greater response of the nitrogen metabolism to dietary protein in older men than in women or gradual failure of kidney function which is more marked in the men.—B. SURE

Abnormalities in serum cholesterol levels, lipoprotein patterns, and chylomicron counts are among the alterations frequently but not routinely found in patients with arteriosclerosis. The following paper confirms a previous report of increasing chylomicronemia with aging. However, the conclusion that serum cholesterol levels may not be related to the pattern of fat absorption does not seem warranted.

The Relation of Age to Fat Absorption in Adult Women Together with Observations on Concentration of Serum Cholesterol. P. Garcia, C. Roderuck, and P. Swanson. *J. Nutrition* 55: 601, 1955.

Information about changes occurring in digestive and absorptive processes with progression of age in man is meager. At the present time, there is increasing interest in lipid metabolism and its relation to the etiology of atherosclerosis. There is evidence that there may be a link between dietary fat or dietary cholesterol or both and the occurrence of this degenerative disease in man. Early workers have demonstrated that chylomicron particles in the blood are related to the fat absorbed from the intestines. Chemical analyses reveal that this fraction of the blood lipoproteins consists chiefly of neutral fat, about 7 per cent protein, small amounts of phospholipid, and contains less than 1 per cent of the total serum cholesterol.

The study was undertaken to investigate the relation of age to the pattern of fat absorption in adult women. Serum cholesterol was also measured. Since both serum cholesterol and the number of chylomicron particles in the blood have been associated with the development of human atherosclerosis, it seemed of interest to find out whether they were related to one another.

It was found that the pattern of fat absorption changes with advancing age. The maximum number of chylomicrons appearing in the blood after a standard test meal increases with advancing age. The longer the period required to reach a peak count, the greater the number of chylomicrons in the blood at the time. Concentrations of serum cholesterol increase with age up to the seventh decade, when a decrease occurs. The peak chylomicron count following a high fat test meal shows the same trend, but no statistically significant relationship was found between serum cholesterol concentrations and the peak chylomicron count. Thus, serum cholesterol may not be related to the pattern of fat absorption. —B. SURE

LIVER DISEASE AND NUTRITION

Fatty infiltration of the liver has been described in the presence of obesity and has been produced by low protein and by high fat diets in animals. The feeding of choline has reduced the fat content of the liver when fat deposition was caused by a high cholesterol intake; it is ineffective when fat infiltration is due to protein restriction. Histological tests can differentiate liver disease due to protein or threonine deficiency from that attributable to choline deficiency. It has been shown by Artrom that the oxidation of fatty acids by the liver is increased by lipotropic substances such as choline, which may account for its effect in instances of fat infiltration produced by certain factors other than protein deficiency. Thus, the lipotropic effect of choline may be mediated through increased fat oxidation, possibly associated with its activity as a methyl donor or phospholipid precursor.

Changes in Chemical Composition During the Development of 'Cholesterol Fatty Livers.' J. H. Ridout, C. C. Lucas, J. M. Patterson, and C. H. Best. *Biochem. J.* 58: 297, 1954.

This is a study of the accumulation of the different lipid components in the livers of rats fed diets containing 0.2–1.6 per cent of cholesterol.

On a percentage basis, the accumulation of lipids in the liver reached an equilibrium after three weeks on the diets; actually, the deposition of glycerides and cholesteryl esters continued throughout the experiment. Glycerides and water accounted for 80–90 per cent of the liver weight increase during the first three weeks, but on continued feeding the relative amount of glycerides deposited decreased and that of water increased. The sum of these two components still accounted for 80 per cent of the weight gain. The accumulation of cholesteryl esters in the livers of cholesterol-fed rats increased 200-fold; however, these esters never accounted for more than a small percentage of the total lipid material deposited. —M. K. HORWITT

Preventive and Curative Studies on the 'Cholesterol Fatty Liver' of Rats. J. H. Ridout, C. C. Lucas, J. M. Patterson, and C. H. Best. *Biochem. J.* 58: 301, 1954.

When the cholesterol intake was moderate (0.2 per cent), the addition of 0.32 per cent choline or an equivalent weight of betaine was able to prevent the excessive deposition of both glycerides and cholesteryl esters in rat liver. When the cholesterol intake was high (1.28 per cent), choline supplementation failed to prevent excessive accumulation of cholesteryl esters. After cholesterol was removed from the diet, vitamin B_{12} had a considerable curative effect on the removal of liver glycerides, but inositol was ineffective. Concentrations of 0.42 per cent of betaine hydrochloride or equimolecular amounts of choline were most active in their curative effect on "cholesterol fatty livers."—M. K. HORWITT

The Lipotropic Activity of Protein. C. C. Lucas and J. H. Ridout. *Canad. J. Biochem. & Physiol.* 33: 25, 1955.

A distinct lipotropic effect of casein has been observed in rats under conditions that appear to preclude any explanation involving the methionine or choline content of the ration. Diets low in protein produce a type of fatty liver that is not prevented by choline and is histologically different from that of choline deficiency. Choline appears less effective in such diets than in ones more adequate in protein. Presumably choline has only a limited effect on abnormal fat in periportal regions because the biochemical basis of this lesion seems to involve faulty formation of lipoprotein due to shortage of essential amino acids.—AUTHORS

Effects of Dietary Protein, Lipotropic Factors and Realimentation on Total Hepatic Lipids and Their Distribution. C. H. Best, W. S. Hartroft, C. C. Lucas, and J. H. Ridout. *Brit. M. J.* 1: 1439, 1955.

In rats fed diets containing a moderate amount of fat and 9 per cent or less protein, supplementary choline failed to prevent completely the accumulation of fat in periportal regions of the liver. Choline deficiency itself caused an accumulation of fat bordering on the central vein of the hepatic lobule. The massive accumulation of hepatic lipids seen in choline deficiency was not reproduced by protein deficiency.

Some rats, when transferred from a low protein diet (3 per cent casein) to an "adequate" diet (containing 18 per cent casein and 0.5 per cent choline chloride), showed a transient fatty liver, the fat appearing in the periportal regions only; the livers returned to normal during 3 weeks on the adequate diet.

The techniques and results are presented in detail and there is a brief discussion on points of possible clinical interest (c.f. previous abstract).—F. E. Hytten

The nutritional requirements for adequate cellular metabolism may be strikingly altered in the presence of infection. The high frequency of liver disease in areas where serious infection and parasitic infestations are common events may reflect a failure to meet these increased requirements.

Disorders of the Liver in Tropical Nutritional Diseases. J. Waterlow. *Proc. Brit. Nutr. Soc.* 13: 135, 1954.

It is now well recognized that liver disease is more common in the tropics than in temperate countries. The author of this paper thinks it is reasonable to attribute this to malnutrition, but he does not regard this as proved. In many instances the relation to diet is not clear-cut, and the lesions and their sequence in man do not always resemble those that have been produced by dietary means in animals. If we leave aside conditions that are undoubtedly caused by infection or infestation, the common types of liver damage may be classified broadly under three heads: fatty liver, fibrosis and cirrhosis, and carcinoma.

The author, who is associated with the Department of Physiology, University College of the West Indies, Jamaica, finds that the nutriture in the Gambia region seems to be no worse than that in many other countries. It was, therefore, difficult to regard this condition, which, by comparison with other places, seemed to be both precocious and severe, as a purely dietary injury. The clinical and epidemiological evidence suggested that a combination of two factors might be involved—malnutrition and malaria.—B. Sure

Fibrosis of the Liver in West African Children. J. H. Walters and J. C. Waterlow. *Med. Res. Council, Spec. Rep. Ser.* No. 285, H.M.S.O., London, 1954.

This monograph describes the natural history of fibrosis of the liver as observed in the Gambia, British West Africa. Biopsy specimens were obtained from 4 groups of cases—infants, mostly malnourished; normal children; children with symptomless hepatomegaly; and children with hepatomegaly together with edema, ascites, or severe anemia. The livers have been classified according to changes in the fibrous tissue. In 45 out of 79 subjects there was a mild periportal fibrosis without distortion of lobular architecture. This took two forms: the commonest was a coarse thickening of portal tracks with a tendency for neighboring tracts to link up—a change very similar to the so-called "stellate fibrosis" described in East Africa. This was found in more than half the children examined. It was neither more common nor more severe in those with symptomless hepatomegaly than in controls. Enlargement of the liver in Gambian children cannot, therefore, be regarded as evidence of a pre-cirrhotic lesion. The clinical evidence suggests that the enlargement is caused by malaria, but how this is brought about is not clear.

The second type of periportal fibrosis was a proliferation of fine reticulin fibers which appeared to be growing irregularly into the lobule from the tracts, accompanied by a marked infiltration of histiocytic cells, giving the appearance of an active lesion. In some of these livers there was mild or moderate fatty infiltration. The severely fatty liver characteristic of kwashiorkor was never seen. Frank necrosis was not found, but there was evidence of piecemeal degeneration of parenchymatous cells. This type of fibrosis was found most often in infants. Clinically, these cases were characterized by weight loss and active malarial infection, often with anemia and sometimes with hypoproteinemia. Edema was not common. Dyspigmentation was sometimes present, but lesions of skin and musosae characteristic of kwashiorkor were seldom found and were never severe.

In seventeen patients fibrosis was diffuse, with distortion of the lobular architecture. The findings suggest that this lesion may result from an extension of the fine periportal fibrosis, and represent a more advanced stage of the same pathological sequence.

Advanced diffuse fibrosis was commonly associated with edema, ascites, or anemia, although some cases with severe fibrotic changes were clinically normal except for enlargement of the liver and spleen. In this group no relation could be established between the presence or severity of fibrosis and any of the individual clinical findings, such as hepatomegaly, splenomegaly, edema, ascites, or anemia. The cause of these phenomena is discussed; the clinical evi-

dence suggests that they are not direct effects of liver damage, but rather that malnutrition and malaria are the most important causal factors in the production of this complex and variable clinical picture.

Analysis of the environmental background shows that these same factors—malnutrition and malaria—have their major incidence and their most weighty impact at just that age in life when the earliest liver lesions are found. No evidence was obtained to suggest that virus hepatitis plays a significant part in causing fibrosis of the liver in the Gambia.

The question is discussed, whether either malaria or malnutrition could be the cause of the liver lesions that have been found. It is concluded that in this particular series neither factor alone can be regarded as a sufficient cause, but both must be implicated. The hypothesis is put forward that malaria causes a localized, essentially inflammatory reaction in the portal tracts. If at the same time there is a nutritional injury to the liver the fibrosis evoked in the first place by malaria tends to spread into the parenchyma. Many infants pass through a phase of malnutrition during the second year of life: at this time the liver shows an active periportal fibrosis. Normally, with an improving nutritional state this lesion heals, leaving nothing but residual scars, which may be regarded as evidence of chronic malarial infection. In a few children, however, as a result of continuing or periodic malnutrition, healing does not occur; the fibrosis progresses and becomes diffuse.

This hypothesis of a dual etiology presupposes the simultaneous action of two stimuli: a nutritional or metabolic injury to the parenchyma tends to provoke a proliferation of reticulin fibres; this injury by itself, however, is not enough to cause a widespread fibrosis unless the fibrous tissue has been made irritable or hyper-reactive by repeated malarial infection.—J. WATERLOW

Elevated levels of serum cholesterol and lipids have long been associated with the nephrotic syndrome. The rise in lipid concentration is related to the intact liver and may involve a more complex mechanism than a decreased rate of degradation, as has been suggested previously.

Role of Liver in Pathogenesis of Experimental Nephrotic Hyperlipemia. W. Heymann and D. B. Hackel. *Metabolism* 4: 258, 1955.

Experimental renal disease produced by antirat kidney serum simulates human nephrosis and is associated with hyperlipemia. In the present study, the role of the liver in the production of hyperlipemia was investigated. Liver damage produced by common bile duct ligation resulted in a less severe form of nephrotoxic renal disease. In animals with established renal lesions, ligation of the bile ducts did not alter the hyperlipemia. In subtotal hepatectomized animals, hyperlipemia was less marked than in the control group after both had received the nephrotoxic serum. The results suggest that the liver is involved in the mechanism that elicits nephrotic hyperlipemia.—C. R. SHUMAN

The variable response of patients in hepatic coma to treatment makes it difficult to accept this favorable report concerning sodium glutamate without further evidence.

Dietetic Aspects of the Treatment of Liver Disease. A. L. Latner. *Proc. Brit. Nutr. Soc.* 13: 139, 1954.

It is important to differentiate coma accompanying acute severe disease from that resulting from a long-standing cirrhosis. In the former instance, there is some chance that the liver cells may regenerate after they recover from the acute noxa. With well-developed Laennec's cirrhosis, even when the patient is comatose, there are relatively few necrotic cells; the parenchymal cells have for long been subject to ischemia which must obviously diminish their powers of regeneration. Dr. Latner has confirmed the claims of Walshe (*Lancet* 264: 1075, 1953) that coma of cirrhosis can be reversed by large doses of sodium glutamate. However, this type of therapy has not been successful in acute cases.—B. SURE

The low 17-ketosteroid and elevated corticoid levels seen in cirrhosis apparently reflect alterations in steroid metabolism associated with liver disease. These changes have also been demonstrated by Samuels (J. Clin. Investigation *33: 1524, 1954*).

Steroid Balance and Testosterone Metabolism in Cirrhosis. J. Warter, J. Schwartz, and J. P. Weill. *Presse méd.* 62: 1019, 1953.

The urinary steroid picture in cirrhotics shows a lowering of the 17-ketosteroids and a moderate rise of the corticoids and phenol-steroids. This picture appears to be specific. Alterations of the adrenal cortex, modifications of urinary elimination, and deficiencies do not account for these findings. Testosterone loading tests show that the endocrine symptoms which frequently appear during the course of cirrhosis are directly connected with the impairment of the hepatic cell.—H. GOUNELLE

Hepatolenticular degeneration is a rare disease characterized by abnormal storage of copper in the liver and basal ganglia, Kayser-Fleischer corneal rings, decreased serum copper and low serum ceruloplasmin levels, and increased urinary excretion of copper, amino acids, and peptides. Increased intestinal absorption of copper and deposition within the tissues, together with renal tubular damage and chelation of copper by the amino acids, may explain the manifestations of the disease.

Hepatolenticular Degeneration: A New Metabolic Disorder. G. Boudin and B. Pepin. *Presse méd.* 62: 243, 1954.

The authors were able to follow and treat three cases of hepatolenticular degeneration. They studied the disturbances in copper metabolism, the urinary amino acids, and the effects of BAL. The anatomical-clinical signs are explained by the disturbances of copper metabolism and the hyperaminoaciduria. The authors were able to demonstrate biological abnormalities in the collateral relatives or descendants of these patients who were free of all clinical signs, thus revealing the existence of subclinical forms of the disease.—H. Gounelle

CHOLINE METABOLISM

The hepatic levels of choline oxidase have been found to be decreased in choline-deficient rats. However, the significance of the level of the enzyme in the liver as a possible factor in the production of fatty infiltration is doubtful. The following paper provides comparative data on the hepatic levels of the enzymes involved in the formation of methionine in several species.

Metabolism of Choline and Related Compounds by Hepatic Tissue from Several Species Including Man. C. J. Kensler and H. Langemann. *Proc. Soc. Exper. Biol. & Med.* 85: 364, 1954.

Data on the activity of choline oxidase, succinoxidase, tributyrinase, and the enzymes concerned in the formation of methionine from choline, betaine or dimethylthetin and homocysteine were evaluated comparatively in liver tissue derived from the rat, mouse, guinea pig, rabbit, chicken, dog, and man. The succinoxidase activity of four samples of fresh human liver was markedly lower than those of the other species. Tributyrinase activity was of the same order in man as in livers from rats and mice. Betaine and dimethylthetin served as methylating agents (for homocysteine) with the livers of all species. Human liver (like that of rabbit and guinea pig) is low in choline oxidase and capacity to utilize choline for methylation of homocysteine.—L. W. Kinsell

The necessity of choline for maintenance of a normal cardiovascular system in rats is confirmed. Choline-deficient animals were found to develop intimal and medial deposits of lipid and calcium salts in the aorta, coronary arteries, and myocardium. These lesions were prevented by choline. However, according to a report by Wissler et. al. (Arch. Path. *57: 333, 1954), a high choline intake may increase atheromatosis. (See also* Am. J. Clin. Nutrition *2: 396, 1954).*

Dietary Choline and the Maintenance of the Cardiovascular System in Rats. G. F. Wilgram, W. S. Hartroft, and C. H. Best. *Brit. M. J.* 2: 1, 1954.

Accumulation of fat in the myocardium, followed by necrosis of the muscle, was produced in rats fed a choline-deficient diet. It was occasionally possible to produce choline deficiency lesions in the absence of dietary fat, suggesting that lack of choline is of basic etiological significance, but the addition of high levels of fat to the diets greatly increased the numbers of animals with affected hearts. Of the triglycerides tested, those of lauric acid produced the highest proportion of cardiac lesions, and of the naturally occurring fats, beef fat and lard resulted in more cardiac damage than corn or coconut oil.

Similar lesions in the liver and kidney also occurred; there were instances of cardiac lesions in the absence of renal necrosis, but none in the absence of fatty lesions of the liver.

On some diets, female rats were more resistant than males, and there were other differences in susceptibility referable to age differences and variations in calorie and protein intake.

The rats were protected from cardiac lesions by supplements of dietary choline.

There is considerable detail in the description of methods and results, and this paper should be read in the original.—F. E. Hytten

Conflicting reports have appeared on the effect of choline and other lipotropic agents upon human arteriosclerosis. So far the evidence favors the view that choline has no beneficial effect upon this disease and is not able to reduce the concentration of serum cholesterol.

Effects of Lipotropic Substances on the Cholesterol Content of the Serum of Rats. J. H. Ridout, J. M. Patterson, C. C. Lucas, and C. H. Best. *Biochem. J.* 58: 306, 1954.

Though the substances that prevent or cure fatty livers are also concerned in the control of serum cholesterol, the authors do not believe that this fact provides any support for the claims that lipotropic substances reduce the concentrations of cholesterol esters in the serum. Also the claim for choline as a therapeutic aid in atherosclerosis "has no firm basis in fact and may serve as a false pathway which diverts energy from the profitable study of atherosclerosis and the real realms of choline action." However, it is hoped that further study of new approaches may reveal a relationship between the lipotropic agents and atherosclerosis.

The time, with respect to the last meal, at which a blood sample is taken is important when studying either lipotropic agents or effects of excessive cholesterol ingestion. Postprandial elevations of serum cholesterol return to normal after about 18–24 hours. (In the absence of choline there is no postprandial elevation.)

In "preventive" studies choline and betaine maintain the serum cholesterol within normal levels but

inositol failed to prevent the characteristic fall. In "curative" studies the replacement of choline and betaine in the diet promptly restores the concentration of serum cholesterol to normal and the effect of vitamin B_{12} is similar.

The experimental findings do not support the claims that lipotropic substances reduce the concentration of bound cholesterol in the serum.—M. K. HORWITT

Choline is absorbed from the intestine by way of the portal vein rather than by the intestinal lymphatics. As much as 50 per cent is decomposed to trimethylamine by intestinal bacteria. This decomposition may be suppressed by the prior oral administration of antibiotics.

Absorption of Choline From Intestinal Loops in Dogs. W. G. Rohse and G. W. Searle. *Am. J. Physiol.* 181: 207, 1955.

Choline chloride (1 gram) was introduced into excised ileal loops and then incubated. Between 1–4 hours there was a loss of 140 mg of choline. During the same period, 10 mg of trimethylamine appeared. Incubation time was not a factor between 1–4 hours. Most absorption took place in the first hour. There was more absorption with higher doses. In Thiry-Vella loops from ileum there was more absorption of choline than was observed in acute ileal loop.—M. J. OPPENHEIMER

Although choline deficiency in the rabbit and guinea pig will result in poor growth, as well as other signs, this effect is not seen in mice.

Effect of Choline-Deficient Diets on Growth, Reproduction and Mortality of Mice. L. Mirone. *Am. J. Physiol.* 179: 49, 1954.

Diets deficient or totally lacking in choline do not alter growth curves of female mice. However, conception rates in choline-deficient mice are lower than in controls. Nonviable newborn may result and mortality is high between 3–4 months. Maternal deaths at parturition may result from profuse bleeding and failure to completely expel the fetus in choline-deficient mice. It is possible that uterine loss of contractility may explain these last findings.—M. J. OPPENHEIMER

ITEMS OF GENERAL INTEREST

Food Waste and Loss of Weight in Cooking. G. N. Chappell. *Brit. J. Nutrition* 8: 325, 1954.

There is little information on the percentage of waste during preparation of food by the average woman. Such figures would be of value in dietary surveys, since some food tables give analyses for raw foods only "as purchased," so that edible portions on dietary records must often be converted to "as purchased" amounts before conversion to calories and nutrients. During two long-term dietary investigations conducted in the Household Arts Department, Queen Elizabeth College, University of London, records of wastage in food preparation were made and results are presented for comparison with existing tables or as a supplement to them. In addition, alterations in weight during cooking are of great importance when the nutritive value of edible portions of food is to be calculated. Alterations are chiefly due to loss or absorption of water or other liquid and also to loss of weight when fat melts and connective tissue hydrolyzes to gelatin. Outer layers of potato may slough away and fish may break apart.

A table of the change of weight of various foods during domestic cooking is presented. Loss of weight varied between 13 and 37 per cent for puff pastry, 7 and 33 per cent for grilled fish, and 13 and 39 per cent for roast meats. Green leafy vegetables generally gained weight on boiling. A list of the wastage of fruits, vegetables, and fish during preparation for the table is given and average values are compared with those in tables of food composition. Unavoidable waste is so great for many commodities that persons handling foods should avoid wastage due to careless preparation.—B. SURE

A Case of Favism. O. R. McCarthy. *Lancet* 1: 748, 1955.

This may be the first recorded description of favism in a person of non-Mediterranean descent. It occurred in a 9-year-old English boy and the case record is given in detail. It is possible that the boy's grand-uncle had the same disease.

"Favism should be considered as a possible cause of haemolytic anaemia and unexplained jaundice, even in patients who are not of Mediterranean stock."—F. E. HYTTEN

Effect of Extirpation of Various Organs on Rat Serum Amylase Levels. G. S. Wiberg, M. W. Little, and J. Tuba. *Am. J. Physiol.* 179: 53, 1954.

Surgical trauma and postoperative anorexia were observed to produce a significant transient reduction in serum amylase. Removal of the gonads or the liver did not influence the serum level of this enzyme. Testosterone propionate had no action on serum amylase levels in castrated rats of either sex. Any observed reduction in males could be ascribed to diminished food intake. An 80 per cent partial pancreatectomy is followed by diabetes and decreased serum amylase activity, and even though there is polyphagia, the serum amylase levels are not returned to normal. High levels of amylase noted after radical pancreatectomy indicate multiple origins for this enzyme. Anterior pituitary growth hormone was demonstrated to influence amylase levels in hypophysectomized rats. Serum levels are correlated with food intake.—M. J. OPPENHEIMER